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THE EFFECT OF CERTAIN DRUGS ON THE RESPIRATION AND GASEOUS METABOLISM IN NORMAL HUMAN SUBJECTS

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The literature on the effects of drugs upon the respiration in normal human subjects is practically confined to the researches of Loewy (1) and Lindhard (2), the former experimenting with morphine, camphor, chloral and alcohol, and the latter with morphine, strychnine and chloral. Both researches were based on a comparison of the respiration when breathing CO₂ rich air, and when breathing normal air, both before and after the administration of the drug. Loewy studied the relation of the volume of expired air and its CO₂ percentage; Lindhard, working about twenty years later, improved on Loewy's method in that he compared the alveolar CO₂ percentage and the volume of the alveolar ventilation. In this paper, we have studied the effect of drugs upon the respiration, confining our observations to changes in the usual respiration factors under conditions of breathing ordinary air, and avoiding the procedure of exerting on the respiratory center an extra stimulus such as is not met with under ordinary circumstances.

In previous papers, Higgins (3) has shown the effect of coffee upon the alveolar carbon dioxide tension, and Edsall and Means (4) have presented and discussed experiments upon the effect of atropine, caffeine, camphor, and strychnine upon the respiration of the normal human subject. This present investigation includes the elaboration and improvement of the methods used in the preceding work and further experiments with the above four drugs and in addition experiments with heroin and morphine.

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We have endeavored to analyze carefully the factors causing respiratory changes due to the action of the drugs and have made observations to detect changes which may occur (1) in the sensitivity of the respiratory center, (2) in the relative amounts of the normal stimulants to the center, (3) in the bronchial musculature, (4) in the rate, depth, and regularity of the respiration, and (5) in the total gaseous exchange. Commonly the term, respiratory stimulation, means an increased volume of air respired in a unit of time, and respiratory depression means a decreased volume. While most workers have made some allowance for change in respiration rate in interpreting an increased respiratory volume, none seem to have considered all the factors involved and their interrelation. The possibilities of changes in the gaseous metabolism and in the bronchial musculature seem to have been especially overlooked. An increased metabolism naturally requires a greater lung ventilation to carry away the increased CO_2 production and a drug causing such a change can hardly be called a direct, but rather an indirect, respiratory stimulant. Any change in the tone of the bronchial musculature, leading to a change in volume for each respiration, as the result of a larger or smaller "dead space," may make considerable variation in a minute's respiratory volume and is a factor which has been generally overlooked by investigators, a changed ventilation following the administration of a drug having been usually attributed solely to action on the respiratory center.

The sensitivity of the respiratory center was followed by making frequent determinations of the alveolar CO_2 tension before and after the administration of a drug. The Haldane method (5) was used, and to avoid any changes in the CO_2 tension which might be due to body position or food (3), all the determinations on any given day were made with the subject in the same position and at least 6 (generally 12) hours after the last meal. The experimental findings of many workers, especially of Haldane and Hasselbalch, have led to the quite general acceptance of the theory, first presented by Winterstein (6) that it is the H-ion concentration of the arterial blood which is the chemical

regulator of respiration. Thus under given conditions a certain H-ion concentration in the arterial blood is necessary to stimulate the respiratory center to induce respiration; if a drug be given which will stimulate (or depress) the center, it will require a lower (or higher) H-ion concentration in the arterial blood to maintain respiration. The decrease (or increase) in the acidity of the arterial blood will be due to less (or more) carbonic acid, if the other acidity of the blood remains unchanged. The alveolar carbon dioxide tension is practically equal to the carbon dioxide tension of the arterial blood. Thus, we have interpreted changes in the alveolar carbon dioxide tension found after the administration of a drug (such changes not ordinarily occurring otherwise) as meaning that the drug had exerted a stimulating effect on the respiratory center if the alveolar CO_2 tension fell, or a depressing effect if it rose.

It seems advisable here to make a statement as to what extent changes in the alveolar CO_2 tension can be accurately ascertained and the degree of their significance. The errors in determining alveolar CO_2 tension at rest—outside of possible small theoretical errors which are constant and do not affect comparisons (7)—are derived practically wholly from abnormal breathing of the subject before the sample is taken, any error in analysis being insignificant. With individuals, as in this investigation, who have had some experience in being subjects for the Haldane determination, this error is reduced to a minimum and the duplicates (i.e. 4 samples) taken within a few minutes of each other very rarely differ more than 1 mm. tension from each other or 0.5 mm. from the average. Changes in the alveolar CO_2 tension which amount to 1 mm. or more are considered positive differences, and due to the superimposed factor if they are found consistently two or more times after the drug. Haldane and his coworkers (8) have shown that an increase in alveolar CO_2 of 0.2 per cent (= 1.4 mm.) is sufficient stimulus to cause doubling the ventilation of the lungs, while a drop of 0.2 per cent leads to apnea; while one must remember that in the course of the day even when fasting, the alveolar CO_2 tension (owing to various causes) changes somewhat irregularly

even as much as 1.5 mm., these figures of Haldane give some idea of the effect of these variations when superimposed, and one can judge roughly the power of the drugs upon the respiratory center in the experiments in this paper.

Another possible cause for a change in the alveolar CO_2 tension would be a change in the acids of the blood (other than CO_2). A change in the blood acidity due to failure of the kidney to functionate properly should be manifested very slowly and gradually. A change in the blood acidity, if due to metabolic causes, would probably be from an increase of acid products as lactic or B-oxybutyric or diacetic acids; if these bodies were formed in sufficient quantities to bring about an effect which might appear in a short time, it would probably be indicated by a significant change in the respiratory quotient. A change in the acidity of the blood was looked for in one experiment, where there was a change in the respiratory quotient, by determining the dissociation of the blood at 17 mm. tension of oxygen and 37°C . by the method of Barcroft (9), but was not found.

To find whether a drug had a broncho-constrictor or a broncho-dilator action, or neither, determinations of the "dead space" of breathing were made before and after the administration of the drug. The "dead space" of breathing represents that portion of each inspiration which does not enter the active interchange of CO_2 and O_2 in the alveoli, but remains in the air passages connecting the alveoli with the air outside the body (mouth, nose, trachea, bronchi, bronchioles). If the "dead space" becomes smaller it is an indication of broncho-constriction, while a larger "dead space" indicates broncho-dilation. The "dead space" was determined by the method of Douglas and Haldane(10). For this purpose, in addition to the alveolar CO_2 determinations mentioned, determinations of the respiratory exchange were carried out either with the Benedict (11) apparatus or with a Tissot spirometer and valves (12) (gas analysis being with the Haldane apparatus(13)). In the latter case knowing the alveolar CO_2 percentage (Alv. CO_2), the volume per expiration (V), and the CO_2 percentages in the expired

(Exp. CO₂), and inspired air (Insp. CO₂), the volume of the "dead space" (D. S.) was calculated from the following formula

$$\text{D.S.} = \frac{(\text{Alv. CO}_2 - \text{Exp. CO}_2) V.}{(\text{Alv. CO}_2 - \text{Insp. CO}_2)}$$

With the Benedict apparatus, the formula became

$$\text{D.S.} = V - \frac{\text{CO}_2}{\text{Alv. CO}_2}$$

where CO₂ is the volume of CO₂ given off per expiration (CO₂ per minute).

$$\frac{\text{CO}_2}{(\text{Resp. Rate})}$$
 The figures given in the tables are at 37°

moist and at the prevailing barometric pressure, correction having also been made for the "dead space" of the apparatus.

In studying with what accuracy the "dead space" may be obtained, it may be noted that errors are possible from the following sources (1) in the determination of the alveolar CO₂ percentage, (2) in the CO₂ percentage of expired air, (3) in the volume per respiration and (4) in the efficiency of the valves. An error of 0.10 per cent in the alveolar CO₂ percentage—a maximum figure for our experiments—will cause an error of about 3 cc. in the "dead space." In the experiments given later with morphine and heroin, very large changes in the "dead space" were found; that these are not the result of incorrect alveolar CO₂ tensions is seen from the fact that in the fourth experiment with J. H. M. on May 5, for the "dead space" to have remained constant (148 cc.), the alveolar CO₂ would have been 7.47 per cent instead of 6.01 per cent and in the seventh experiment of November 8 with J. H. M. for a constant "dead space" of 145 cc. the alveolar CO₂ would have been 6.88 per cent instead of 5.80 per cent; that such errors in the alveolar CO₂ percentage should occur is quite impossible. The expired CO₂ percentage should be accurate, allowing both for analysis and sampling, to ± 0.04 per cent, an amount corresponding to about 3 cc. error in the "dead space." The volume per respira-

tion is accurate in our experiments to well within 2 per cent, which would affect the "dead space" correspondingly not over 3 cc. In experiments, the valves must be working properly, especially the expiratory valve, or expired air would be re-breathed, leading to an incorrect figure for the calculated "dead space." Care has been used in each of these experiments to make sure the valves were tight, and especially when the results obtained show changed "dead space." Thus we have come to the conclusion that in these experiments a difference of 10 cc. in the calculated "dead space" is positive indication of a change in its volume, and when such a change is consistent, is the result of a superimposed factor.

While other explanations of a changed "dead space" are theoretically possible, that it is due to broncho-dilation or broncho-constriction seems the most probable. A change in the number of functioning bronchioles does not seem likely. In any event, it is clear that the net result is the same, an increased "dead space" indicating an increased volume of respiration per unit of carbon dioxide eliminated.

The respiration rate and depth as well as a graphic record of the breathing have been obtained in connection with the experiments with the Tissot apparatus by means of a pneumograph connected to a tambour, giving a record on a smoked drum.

The gaseous metabolism was obtained in 10 minute periods by the Tissot method or the Benedict universal apparatus, both before and after the drug. Outside of the bearing on the metabolism *per se*, it was also important in its relation to the respiration. For an increased gaseous exchange due to a stimulated metabolism, would naturally cause increased respiratory volume (a thing which might from some methods of experimentation be interpreted as stimulation of the respiratory center, whereas it is merely a normal reaction). In connection with the respiration experiments, the pulse rate was followed, which has proved interesting in its relation to the metabolism.

The usual routine of an experiment was as follows. The subject came to the laboratory in the morning breakfastless and lay on a couch quietly for 30 minutes, before any experiment-

ing was done. Alveolar CO_2 determinations (from an average of two to four samples) and respiration experiments were made alternately. After two or three normal experiments, the drug was given and further alveolar air determinations and respiration experiments made during the next 2 or 3 hours. The experiments were with the subject in complete muscular repose (i.e. no voluntary movements). A few of the experiments were made in the afternoon, the subject having had nothing to eat since 7.00 a.m.; otherwise the routine was the same. Three of the experiments reported consisted only of alveolar CO_2 determinations before and after the drug while the subject was sitting in a chair.

In our experiments, we have given the drugs in such doses as are ordinarily used in therapeutics. We were our own subjects² in all but one experiment; Dr. W. W. Palmer of the Massachusetts General Hospital kindly acted as subject in one of the morphine experiments. It is hardly the hope in this paper to cover all the idiosyncrasies incident to the use of these drugs, nor have we experimented with varying doses, but we have endeavored to find the general tendencies resulting from the drugs. Naturally it would be advisable to extend this investigation to pathological cases, and it is our intention to do so.

The drugs selected are those ordinarily used as respiratory stimulants, including atropine, caffeine, camphor, and strychnine, and those as respiratory depressants, including morphine and heroin.

ATROPINE

Heinz (14), (15) reports that with rabbits, atropine did not appreciably change the respiratory volume. Wood and Cerna (16) found with dogs that atropine stimulated the respiration; these conclusions were drawn from an increased rate and minute volume of respiration.

The experimental work on the effect of atropine on the respiration has dealt chiefly with two problems, namely the use of atropine to counteract the depressing effect of morphine

² J. H. M., 76 kg., 175 cm.; H. L. H., 64 kg. 172 cm.; W. W. P., 92 kg. 187 cm.

on the respiration and its action on the nerve fibers of the vagus. Unverricht and Orłowski (17), (18), (19) state that atropine merely augments the respiratory depressant action of morphine; other investigators, Binz (20), Heinz (15), Heubach (21) and Vollmer (22), differ emphatically from them, reporting a counter action of atropine to morphine in increased respiratory volume and deeper and stronger respirations. Broncho-dilation, due to paralysis of the broncho-constrictor fibers of the vagus nerves, is reported by Einthoven (23), by Beer (24) and by Brodie and Dixon (25).

Kelemen (26) using enormous doses of the drug ($\frac{1}{2}$ gram per kilo) found the gaseous metabolism in a curarized animal diminished 7 per cent with the drug, a parallel diminution of the carbon dioxide of the blood occurring. Edsall and Means (4) reported increased respiratory exchange, with diminished calculated alveolar carbon dioxide tension with two human subjects.

In our two experiments with atropine, the classical actions of the drug were noticed in both cases; there was a slowing of the pulse for about 20 minutes followed by an elevation lasting about $1\frac{1}{2}$ hours; the usual dryness of the throat lasting for an hour or so occurred.

In neither of the two experiments does the alveolar CO_2 tension change after giving the drug, at least not consistently, either up or down; so we may conclude that atropine does not alter the sensitivity of the respiratory center. In the subject J. H. M., the "dead space" increases from an average of 157 cc. for the experiments before atropine to 167 cc. for the three last experiments following atropine. The rise with H. L. H. is more marked and decisive, being from 123 cc. before to 142 cc. after. This corresponds to the dilation of the bronchial musculature in animals noted by several investigators. The increased gaseous metabolism noticed by Edsall and Means is apparent also in these experiments. In the experiment with J. H. M., it is very interesting to note the parallelism of the pulse rate and the total respiratory exchange; a drop immediately follows the giving of the drug, but in both cases it is succeeded by a rise. The

Atropine

TIME	ALVEOLAR CO ₂ TENS. <i>mm.</i>	CO ₂ PRODUCED PER MIN. <i>cc.</i>	O ₂ ABSORBED PER MIN. <i>cc.</i>	R. Q.	PULSE RATE	RESPIRATION RATE	VOLUME EX- PIRED AIR		DEAD SPACE <i>cc.</i>
							per min. <i>liters</i>	per resp. <i>cc.</i>	

Subject J. H. M. April 14, 1914

7.30 a.m.	Last meal previous to experiment.								
3.25 p.m.	Lay on couch, to begin quiet period; did not sit up until end of experiment.								
3.36	40.7								
3.47-4.00		195	266	0.735	64	10.5	5.00	476	152
4.06-4.19		195	266	0.735	66	11.0	5.25	475	161
4.35	39.2								
4.44	1 milligram atropine sulphate injected right arm.								
4.46-5.00		182	244	0.750	57	11.3	4.99	442	149
5.05	39.2								
5.18-5.30		202	275	0.735	66	12.6	5.75	457	173
5.36	40.3								
6.06-6.19		205	276	0.745	69	12.8	5.77	451	167
6.20	39.3								
6.47-6.59		199	265	0.755	63	12.8	5.74	448	163

Subject H. L. H. May 27, 1914

7.25 a.m.	Lay on couch, to begin quiet period; did not sit up until end of experiment.								
7.45	40.8								
7.50	40.3								
8.01-8.17		191	253	0.755	78	13.4	5.08	379	124
8.20	39.7								
8.25-8.37		179	237	0.760	74	14.0	4.93	352	121
8.40	39.9								
8.41	1 milligram atropine sulphate injected left arm.								
8.57	40.0								
8.58-9.13		191	242	0.790	73	13.2	5.07	383	128
9.22	40.9								
9.31-9.46		192	248	0.775	94	13.2	5.32	402	145
9.49	39.0								
10.01-10.12		193	251	0.770	95	14.2	5.54	391	147
10.13	39.6								
10.29-10.41		175	229	0.765	72	16.5	5.56	337	149
10.45	39.6								

respiration rate may increase after atropine, but so little that it is hardly significant. The regularity of the respiration was unchanged.

A stimulation followed by a depression of certain fibers of the vagus nerves seems indicated first by the fall and then the rise of the pulse rate and the parallel diminution of the "dead space" (broncho-constriction) and the subsequent increase (broncho-dilation), especially in the case of J. H. M., as seen in comparing the first and last three experiments after giving the atropine.

In conclusion, it seems that any respiratory stimulation due to atropine is to be explained by (1) the increased "dead space," or dilation of the bronchi and (2) increased metabolism. The metabolism being increased, the respiratory volume increases to rid the body of the CO_2 produced, and for a unit of CO_2 eliminated, the respiratory volume increases because of an increased "dead space."

CAFFEINE

Caffeine has been very generally recognized as a stimulant of the respiration and its use in alcohol and opium poisoning is common. Binz (27) and Cushny (28) report an increased respiration rate in animals after caffeine injections; Cushny found the depth unchanged. Heinz (14) and Impens (29) report both increased rate and depth after caffeine with animals. Higgins (3) found that coffee, presumably because of its caffeine content, lowered the alveolar CO_2 tension. Pal (30) found that coffee in cases of muscarine poisoning had a broncho-dilator action. The gaseous metabolism after caffeine has been shown to increase in animals by Hoppe (31) and Heerlein (32) and more recently in men by Edsall and Means (4). In some unpublished experiments in the Nutrition Laboratory, a similar increase (10 per cent) was found after coffee.

In our experiments, the alveolar CO_2 tension fell significantly in every case; in three the drop was almost immediate, while in the other it appeared in about an hour. The respiration rate increased in the two experiments in which it was observed. The gaseous metabolism increased markedly with J. H. M.;

Caffeine

TIME	ALVEOLAR CO ₂ TENS. <i>mm.</i>	CO ₂ PRODUCED PER MIN. <i>cc.</i>	O ₂ ABSORBED PER MIN. <i>cc.</i>	R. Q.	PULSE RATE	RESPIRATION RATE	VOLUME EX- PIRED AIR		DEAD SPACE <i>cc.</i>
							per min. <i>liters</i>	per resp. <i>cc.</i>	

Subject J. H. M. May 22, 1914

8.20 a.m.	Lay on couch, to begin quiet period; did not sit up until end of experiment.								
8.44-9.01		190	244	0.780	56	12.6	5.07	404	145
9.05	41.1								
9.11-9.23		186	244	0.765	58	13.0	5.00	385	147
9.25	42.5								
9.35	42.2								
9.43	0.32 gram caffeine sodium benzoate injected left arm.								
9.53	37.4								
9.56-10.08		221	281	0.785	53	15.8	6.13	389	128
10.12	39.0								
10.17-10.27		210	282	0.745	50	16.3	6.05	373	143
10.30	40.0								
10.39-10.51		201	277	0.725	52	15.3	5.90	385	158
10.53	40.6								
11.07-11.18		210	278	0.755	54	13.4	5.67	423	146
11.20	39.0								

Subject H. L. H. June 2, 1914

8.20 a.m.	Lay on couch, to begin quiet period, did not sit up until end of experiment.								
8.45	41.2								
8.56-9.10		190	237	0.810	73	12.9	4.91	380	120
9.15	40.1								
9.16-9.29		180	224	0.800	66	12.5	4.70	377	117
9.35	40.0								
9.40	0.32 gram caffeine sodium benzoate injected left arm.								
9.44-9.58		192	234	0.820	62	12.5	5.06	406	122
10.00	38.0								
10.09-10.21		193	235	0.825	61	15.7	5.61	358	126
10.25	38.4								
10.40-10.53		205	246	0.835	67	17.3	6.33	367	145
10.55	37.3								
11.18-11.30		196	247	0.795	62	15.5	5.89	379	140
11.32	37.9								

Caffeine

TIME	ALVEOLAR CO ₂ TENS. mm.	TIME	ALVEOLAR CO ₂ TENS. mm.
<i>Subject J. H. M. March 28, 1914</i>		<i>Subject H. L. H. March 30, 1914.</i>	
9.25 a.m.	Reclined in Morris chair.	9.30 a.m.	Lay on couch.
10.20	41.3	9.50	38.9
10.45	42.0	10.30	39.8
10.55	0.5 gram caffeine sodium salicylate injected left leg.	10.35	0.4 gram caffeine sodium salicylate injected left arm.
11.05	40.7	10.50	37.4
11.30	41.4	11.15	38.1
11.57	38.9	11.35	38.9
12.25 p.m.	39.8	12.35 p.m.	38.3
12.55	38.8	1.10	38.2

a parallel rise in pulse did not occur. With H. L. H. an increase in the metabolism seems to have occurred, but it is not large. The "dead space" showed a rise with H. L. H.; with J. H. M., the "dead space" first decreased, and then increased, and in general the variations were irregular.

In two experiments (H. L. H., June 6, and J. H. M., May 5) caffeine was given to study its action after morphine and heroin. In the former case we find the caffeine giving a broncho-dilator effect, and little, if any, effect on the respiratory center, while in the latter, there is the usual stimulating effect on the center and no broncho-dilator action.

In conclusion, it appears that caffeine is a respiratory stimulant because of its effect on the respiratory center. It also seems occasionally to have a broncho-dilator effect. It increases the gaseous metabolism and the respiration rate.

CAMPHOR

Loewy (1), giving a 0.5 gram dose of camphor to two men, reported that with one, there was no change in the excitability of the respiratory center, while with the other, there was a slightly increased excitability; his method, as previously stated,

involved a comparison of the relation of the expired per cent CO_2 to the volume of the expired air.

Impens (29) with rabbits found the respiration unaffected by as much as 1 gram of camphor; a larger amount led to spasms and thus reflexly to an increased respiration. Lewin (33), using rabbits with cut vagi, found camphor made the respiration a trifle slower and somewhat deeper and concluded that camphor stimulated the respiration.

Edsall and Means (4) found a temporarily increased metabolism in one case after camphor and no change in another. Also in one case they found a small drop in the calculated alveolar CO_2 tension, but not in the other.

In the experiments we report, the two subjects show a somewhat different reaction to camphor. In the case of J. H. M., one finds no change in the alveolar CO_2 tension or in the size of the "dead space;" there is an increased metabolism and possibly a fall in the respiration rate. The fall in the respiratory quotient to 0.675 in one experiment is quite surprising, and therefore the experiment was repeated, and the second time no such drop was found. With H. L. H., the alveolar CO_2 tension, the metabolism and respiration rate show little or no change. The only striking effect is in the size of the "dead space;" this increase in size may be due to reflex action from the odor of camphor upon the bronchial musculature, a condition similar to that found in asthma, where often a substance having a distinct sharp smell will relieve the asthmatic attack. This increased "dead space" may account for the drop in the calculated alveolar air as obtained by Edsall and Means.

In general, one may conclude that camphor is by no means certain to stimulate the respiration, and when the respiration is stimulated, it is due to increased "dead space" (bronchodilation) and not to increased sensitivity of the respiratory center. The total metabolism is sometimes increased by camphor injection, but hardly more than 10 per cent. The respiration rate is likely to show a small decrease after camphor; the nature of the respiration as shown by the pneumograph is unchanged.

Camphor

TIME	ALVEOLAR CO ₂ TENS. <i>mm.</i>	CO ₂ PRODUCED PER MIN. <i>cc.</i>	O ₂ ABSORBED PER MIN. <i>cc.</i>	R. Q.	PULSE RATE	RESPIRATION RATE	VOLUME EX- PIRED AIR		DEAD SPACE <i>cc.</i>
							per min.	per resp.	
							<i>liters</i>	<i>cc.</i>	
<i>Subject J. H. M. April 24, 1914</i>									
7.30 a.m.	Last meal previous to experiment.								
2.05 p.m.	Lay on couch, to begin quiet period; did not sit up until end of experiment.								
2.18	38.5								
2.21-2.36		196	270	0.730	62	12.5	5.63	449	162
2.42	39.0								
2.48-3.00		192	273	0.705	58	11.6	5.33	458	150
3.10	37.7								
3.18	2 cc. (0.4 gram) camphor injected left arm (followed by ca. 5 minutes' massage).								
3.28-3.43		207	288	0.720	62	11.4	5.53	485	150
3.46	39.0								
3.57-4.10		201	295	0.680	63	10.2	5.26	513	152
4.20	38.9								
4.34-4.52		201	299	0.675	62	9.7	5.21	535	158
5.00	39.4								
5.05-5.17		205	283	0.725	60	11.7	5.58	475	160

Subject H. L. H. November 28, 1914

8.20 a.m.	Lay on couch, to begin quiet period; did not sit up until end of experiment.								
8.45	38.9								
8.52-9.05		211	237	0.890	66	14.7	5.82	396	127
9.05	39.9								
9.17-9.30		203	234	0.870	66	14.3	5.41	378	114
9.35	40.0								
9.49	2½ cc. (0.5 gram) camphor injected left arm (followed by ca. 5 minutes' massage).								
10.07	39.5								
10.10-10.22		208	242	0.860	65	13.4	5.48	409	117
10.25	39.4								
10.35-10.47		199	236	0.840	62	13.4	5.48	409	133
10.55	39.5								
11.05-11.17		199	240	0.825	61	13.8	5.53	401	135
11.25	39.9								
11.28-11.40		206	242	0.850	63	14.4	5.78	401	139
11.45	40.2								

Camphor—Continued

TIME	ALVEOLAR CO ₂ TENS. <i>mm.</i>	CO ₂ PRODUCED PER MIN. <i>cc.</i>	O ₂ ABSORBED PER MIN. <i>cc.</i>	R. Q.	PULSE RATE	RESPIRATION RATE	VOLUME EX- PIRED AIR		DEAD SPACE <i>cc.</i>
							per min. <i>liters</i>	per resp. <i>cc.</i>	
							<i>Subject J. H. M. December 15, 1914</i>		
8.40 a.m.	Lay on couch to begin quiet period; did not sit up until end of experiment.								
9.15	40.2								
9.22-9.35		205	255	0.805	—	12.2	5.25	430	138
9.40	41.5								
9.45-9.57		204	250	0.820	52	12.5	5.29	424	143
10.00	41.0								
10.05-10.17		203	258	0.790	53	13.5	5.41	401	137
10.20	40.0								
10.25	2½ cc. (0.5 gram) camphor injected left arm.								
10.40	40.2								
10.44-10.57		204	267	0.765	57	12.5	5.23	418	131
11.00	40.7								
11.10-11.23		207	262	0.790	55	12.7	5.29	416	135
11.30	41.4								
11.34-11.46		213	258	0.825	57	13.0	5.62	432	152
11.50	40.8								
11.57-12.09		217	275	0.790	56	12.9	5.56	431	136
12.10	40.0								

STRYCHNINE

The literature on the effects of strychnine on the respiration shows diversity of opinion as to whether this drug is a stimulant or not. Wood and Cerna (16) report that strychnine injected subcutaneously increased the rate and depth of respiration in dogs ordinarily and especially when they had been diminished by chloral. Biberfeld (34) found that an injection of 0.001 gram strychnine in a rabbit did not change the respiration rate significantly; if the animal was under morphine, however, the rate was increased. Cushny (28) with rabbits found strychnine increased the rate, but rendered the depth of respiration more shallow, so long as no spasms occurred; but in spasms, the depth as well as the rate increased, due in part,

Cushny says, to increased production of CO_2 by the muscular activity, but also to increased excitability of the respiratory center; however, amounts that produce spasm are naturally much in excess of the therapeutic dose. Impens (29) found in rabbits strychnine in doses up to 0.38 mg. per kilo did not distinctly influence the respiration, while that dose was sufficient to increase the rate and depth for but a short time. Lindhard (2) with himself as subject found 0.006 gram strychnine nitrate increased the excitability of the respiratory center. Edsall and Means (4) found no effects from strychnine on the respiration and gaseous metabolism.

Of the five experiments we report, those with H. L. H. on November 5, and with J. H. M. on December 31, are clearly negative throughout, while the other three experiments are also negative in most of the points studied. Thus in no case, is the rate and regularity of the breathing, or the gaseous metabolism, changed. The alveolar CO_2 tension is clearly unaffected by the drug, except perhaps in the experiments of March 31 and November 3; in the former case a small drop is apparent for a half hour after the injection; in the experiment of November 3, the rise in the CO_2 tension seems to have begun before the injection and can hardly be ascribed to the drug. The "dead space" while unchanged in the experiments of November 5 and December 31, seems to fluctuate considerably on November 19, and especially on November 3. On November 3, the rise in the third period from 136 cc. to 178 cc. was so great as to lead to a suspicion of possible error in experimenting; therefore, we repeated the experiment twice, failing to obtain any indication of a similar difference. The rise in the last two periods on November 19 of about 15 cc. over the control periods would be significant if confirmed in other experiments; but since it is not, one must hesitate to ascribe the rise to the drug, the more so because the rise in "dead space" on November 3 is immediate while on November 19, it does not occur for over one hour.

In general strychnine does not affect the gaseous metabolism, the respiration rate and regularity, or the sensitivity of the respiratory center. Sometimes it appears to cause bronchodilation.

Strychnine

TIME	ALVEOLAR CO ₂ TENS. mm.	CO ₂ PRODUCED PER MIN. cc.	O ₂ ABSORBED PER MIN. cc.	R. Q.	PULSE RATE	RESPIRATION RATE	VOLUME EX- PIRED AIR		DEAD SPACE cc.
							per min. liters	per resp. cc.	

Subject H. L. H. November 5, 1914

8.00 a.m.	Lay on couch, to begin quiet period; did not sit up until end of experiment.								
8.50	40.9								
8.57-9.08		203	239	0.850	73	14.5	5.25	363	116
9.15	40.2								
9.20-9.31		203	237	0.855	68	15.1	5.24	348	107
9.35	40.2								
9.41	4.5 milligrams strychnine sulphate injected left arm.								
9.50	39.8								
9.53-10.04		196	236	0.830	68	14.4	5.12	355	111
10.10	39.7								
10.19-10.29		208	246	0.845	70	14.0	5.25	374	109
10.35	40.3								
10.44-10.55		202	243	0.830	71	13.8	5.04	365	103
11.00	39.9								
11.14-11.25		195	236	0.825	69	12.4	4.82	389	105
11.35	39.2								

Subject J. H. M. November 3, 1914

8.35 a.m.	Lay on couch to begin quiet period; did not sit up until end of experiment.								
8.50	40.8								
9.02-9.13		207	255	0.810	64	11.1	5.13	463	129
9.20	39.7								
9.24-9.35		208	258	0.805	60	11.8	5.26	446	136
9.40	42.0								
9.55	4.5 milligrams strychnine sulphate injected left arm.								
10.02	43.1								
10.05-10.16		202	255	0.790	57	10.9	5.25	481	178
10.20	42.8								
10.29-10.41		208	260	0.800	55	11.6	5.27	454	154
10.50	42.1								
11.01-11.12		202	254	0.795	54	12.3	5.20	422	144
11.20	41.9								
11.31-11.42		207	262	0.790	58	13.0	5.47	421	139

Strychnine—Continued

TIME	ALVEOLAR CO ₂ TENS. <i>mm.</i>	CO ₂ PRODUCED PER MIN. <i>cc.</i>	O ₂ ABSORBED PER MIN. <i>cc.</i>	R. Q.	PULSE RATE	RESPIRATION RATE	VOLUME EX- PIRED AIR		DEAD SPACE <i>cc.</i>
							per min. <i>liters</i>	per resp. <i>cc.</i>	
<i>Subject J. H. M. November 19, 1914</i>									
8.30 a.m.	Lay on couch to begin quiet period; did not sit up until end of experiment.								
9.05	40.5								
9.09-9.20		196	244	0.805	53	13.7	5.33	389	136
9.25	41.3								
9.30-9.40		196	246	0.800	51	13.6	5.23	385	128
9.45	40.4								
9.50-10.01		194	245	0.790	53	13.0	5.23	403	138
10.08	41.3								
10.13	4.5 milligrams strychnine sulphate injected left arm.								
10.20	40.4								
10.22-10.34		199	247	0.810	53	13.2	5.26	399	128
10.40	40.8								
10.47-11.00		207	252	0.820	53	14.5	5.52	382	128
11.10	41.2								
11.20-11.31		207	256	0.810	53	13.9	5.64	405	146
11.50	41.6								
11.52-12.04 p.m.		190	247	0.770	53	12.7	5.25	415	149
12.10	39.5								
<i>Subject J. H. M. December 31, 1914</i>									
8.55 a.m.	Lay on couch to begin quiet period; did not sit up until end of experiment.								
9.30	40.9								
9.45-9.57		205	260	0.785	58	11.7	5.18	444	135
10.00	41.4								
10.07-10.20		201	265	0.760	56	11.8	5.04	426	129
10.22	41.9								
10.25	4.5 milligrams strychnine sulphate injected left arm.								
10.37	41.1								
10.41-10.54		203	266	0.765	57	12.3	5.24	426	137
10.55	41.5								
11.05-11.17		204	264	0.755	56	12.3	5.23	427	131
11.25	39.9								
11.43-11.56		197	261	0.755	55	12.8	5.10	397	125
12.05	42.2								
12.07-12.20		198	262	0.755	57	11.5	5.04	437	138
12.25	40.4								

*Strychnine**Subject J. H. M. March 31, 1914*

TIME	ALVEOLAR CO ₂ TENS.
	<i>mm.</i>
7.30 a.m.	Last meal previous to experiment.
2.25 p.m.	Sat in chair.
2.45	39.9
3.05	38.8
3.10	4.5 milligrams strychnine sulphate injected left arm.
3.18	37.8
3.30	38.0
3.55	38.8
4.25	38.0
4.45	39.5
5.15	38.5

MORPHINE AND HEROIN

As morphine and heroin are so similar in their action and are so closely related, it seems advisable to consider them together here. That morphine in large enough doses is a "respiratory poison" is pretty well established clinically, as is shown by cases of resuscitation solely by artificial respiration. The action of the drug when given in therapeutic doses may however be quite different; it is the effect of such doses on the respiration that is studied in this research. The experimental observation of a diminished respiration rate in animals has been reported by Gscheidlen (35); Leichtenstern (36) in animals noticed diminished depth as well as rate of respiration. Filehne and Kionka (37) reported in morphinized rabbits, that the blood was richer in CO₂ and poorer in O₂, and ascribed it to respiratory depression. Loewy (1) in two human subjects found that the ventilation of the lungs did not increase so much on breathing air containing CO₂ after giving morphine as it did before. Lindhard (2) comparing the relation of the alveolar CO₂ tension and alveolar ventilation, found that after morphine the alveolar ventilation did not increase so much as normally for a definite increase in the alveolar CO₂ tension on breathing a CO₂ containing gas mixture. Cushny (28) reports in experiments on

rabbits a diminution of the respiration rate after morphine which was generally not counterbalanced by an increased depth; also he gives experiments to show that on breathing CO_2 mixtures the respiration was not increased so much after morphine as before and therefore he concludes that morphine was a depressant to the respiratory center.

Brodie and Dixon (25) found with animals that small doses of morphine caused a slight widening of the bronchioles, when tonus existed; with large doses there was at first a dilation but this was soon followed by a significant constriction.

Loewy (38) compared the oxygen consumption of two human subjects in normal sleep and in morphine sleep; in both cases there was a small drop with the morphine, the oxygen consumption per minute falling from 204.65 cc. to 197.95 cc. with one, and from 240.35 cc. to 227.84 cc. with the other. His conclusion is that morphine does not materially influence the respiratory exchange. Böck and Bauer (39) found a diminished CO_2 production and O_2 consumption in a dog in morphine sleep, while in a cat where morphine acted as an excitant, the gas exchange greatly increased. Fubini (40), giving fairly large doses of morphine, found a 50 per cent decrease in the metabolism of dogs and rabbits, a smaller decrease in that of guinea pigs, no change in that of a dove and rise of metabolism in a rat.

Dreser (41), (42) is the first investigator to report experiments with heroin; he claimed that therapeutic doses of heroin, like morphine, were beneficial for a cough, but that while the respiration rate did diminish, yet, unlike morphine, heroin did not reduce the sensitivity of the respiratory center. He came to this conclusion from experiments with rabbits, finding that while heroin lowered the respiration rate, the depths of the individual respirations were correspondingly increased after taking the drug. In other experiments an animal rebreathed air, in one case (with high per cent O_2) to get the accumulation of CO_2 necessary to double the volume of a single respiration, and in another, where the CO_2 was absorbed as fast as exhaled, to find how low the oxygen percentage in the inspired air became before the respiration volume was doubled; the results obtained

with these tests after giving heroin were not different from earlier controls. Thus Dreser's argument for the advantage of heroin over morphine was that the former weakened the effect of mechanical stimulation to the center, but did not change the effect of chemical stimulation (CO_2 and oxygen-want).

Dreser's experiments were not agreed with generally and several controversial papers followed; probably the most significant of these was by Fränkel (43), who repeated some of Dreser's experiments with heroin obtaining the same results, but found that morphine also when given in small doses gave the same results as heroin. Impens (44) made experiments with heroin, and found that after the latter drug, breathing 3 per cent CO_2 caused increase of the volume of a single respiration just as it did before the drug, while after morphine the CO_2 did not increase the respiration at all. Winternitz (45), (46), however, with men, using the method of Loewy maintained that heroin was a respiratory depressant just as much as morphine, the increase in volume for each per cent increase in expired air being less after heroin than before.

Dreser found heroin caused a decrease of 25 per cent in the gaseous metabolism of a rabbit. Winternitz found a diminution of the gaseous exchange with man, his figures being

	CC. PER MINUTE		RESP. QUOT.
	CO_2 produced	O_2 consumed	
Before heroin (0.007 gram).....	162.2	202.7	0.80
$\frac{3}{4}$ hour after heroin.....	161.5	194.4	0.83

Four experiments were made by us with morphine (3 subjects) and two with heroin. The subjective effects of these drugs with the different individuals were not the same. With H. L. H., neither heroin nor morphine caused any subjective effects, except that after morphine, the subject felt more tired than usual at the end of the day. With J. H. M., there was some drowsiness during the experiments, and on getting up from the couch after the experiment, the subject felt very nauseated and

generally indisposed the rest of the day. With W. W. P., there was some drowsiness soon after giving the drug, but this passed off; about an hour after the experiment the subject began to feel a reaction in loss of appetite while eating and slight nausea and marked indisposition which lasted the rest of the day. In all of the experiments the subjects remained awake.

The alveolar CO_2 tension shows a rise in the two experiments with H. L. H., in the experiment with W. W. P., and in the heroin experiment with J. H. M. In the morphine experiments with J. H. M., no rise is apparent. The rise is usually about 2 mm., which is in general not so large as the drop found with caffeine.

The "dead space" in the case of J. H. M. shows in each of the three experiments a decided diminution, of 32 cc. in the experiment of November 8, of 14 cc. in that of June 5, and of 41 cc. in that of May 5. With H. L. H., with morphine, there is no appreciable change (average increase of 7 cc.) but with heroin there is a decided diminution of 20 cc. With W. W. P.³ there is no change in the average figures. In the two cases where there is no change, it seems that before giving the drug there must have been some considerable degree of constriction, because the control "dead space" is so small as compared with other experiments including those in this paper.

The respiration rate was increased one or two per minute by the morphine in several of the experiments, but in no case did either the heroin or morphine cause a slower rate. The type of respiration as shown from the pneumograph record was unchanged.

The pulse rate became a little slower in all but one case after the drug was given; this slowing was noticeable during the last respiration experiment, where the rate was, on an average, about 8 beats lower than in the control.

The total gaseous exchange (heat production) as judged from the oxygen consumption in general seems to be unchanged by

³ That the "dead space" of so large a man as W. W. P. should be so low seem extraordinary; that the figures are correct seems to be shown by determination on another day when an average "dead space" of 115 cc. was found.

these drugs. With J. H. M. the last two experiments after the morphine on November 8, and the last two after the heroin on May 5 show a drop of 5 to 10 per cent in the oxygen, while several of the experiments on June 5 after morphine seem to indicate a high oxygen consumption, but aside from these cases there is no marked difference between the periods before and after the drug was given.

The drop in the CO_2 elimination per minute after giving morphine is very evident, and except with H. L. H. with morphine on June 6, is considerable. This has contributed to the diminution in the ventilation per minute and per respiration. With the drop in the CO_2 elimination, we find in every experiment a fall in the respiratory quotient. This fall in respiratory quotient may be theoretically accounted for in two ways (1) the accumulation or storage of CO_2 in the body and (2) a change in the metabolism, as conversion of fat into carbohydrates. The former was one reason mentioned by Gréhan (47) to account for a drop in the CO_2 elimination in a 15.6 kg. dog from 0.348 gram per minute awake to 0.123 gram per minute in morphine sleep. If there were in our experiments a storage of CO_2 , there may be cited two possible causes for its occurrence (1) a less sensitive respiratory center leading to accumulation of CO_2 in the arterial blood and correspondingly in the venous blood and tissues, and (2) a sluggish circulation leading to storage of considerable amounts of venous blood in the splanchnics. If the former is the case, it should be indicated by increased CO_2 tension in the alveolar air, for an increased alveolar CO_2 tension would signify increased (stored) quantities of CO_2 in the arterial blood and consequently throughout the body. The rise in the alveolar CO_2 tension with morphine is equal to or less than the drop with caffeine, and any drop in respiratory quotient with morphine due to storage of CO_2 should be equivalent to a similar rise in respiratory quotient due to caffeine. But the respiratory quotients in the experiments with caffeine show a slight increase in one case and a fall in the other. Also the largest drop in the respiratory quotient occurs in morphine experiments with J. H. M. in which there was no rise in the alveolar CO_2

tension. The fall in respiratory quotient in the experiments with H. L. H. and possibly with W. W. P. might, however, be ascribed to this cause. It does not seem likely that a slowing of the blood circulation is the cause for the lowered CO_2 output, for then one would expect the O_2 elimination to be correspondingly lessened, with no change in the respiratory quotient.

The lowered respiratory quotients might also be explained by a change in the character of the metabolism as from incomplete combustion (for example, formation of acetone bodies from fat) or by changing of fat to sugar. These two possibilities were studied in the experiment with W. W. P. The acetone bodies being largely acids, should, it would seem, if present, cause the acidity of the blood (exclusive of CO_2) to increase; so an experiment was made to see if the acidity of the blood changed as a result of the injection. This was done by the method of Barcroft (48) in which the O_2 combining power of the blood is studied at 17 mm. O_2 tension. One sample of blood was taken before the injection and one $1\frac{1}{2}$ hours after the injection of the morphine, defibrinated and each exposed to 17 mm. O_2 tension at 37°C . The sample taken before the injection was 60 per cent saturated with oxygen, and that after it 61 per cent saturated. As an increase of acid would have been indicated by diminished saturation, the acidity of the blood was unchanged. Blood sugar determinations by Miss E. B. Babcock of the Nutrition Laboratory were also made on the blood of W. W. P., before and after morphine was given, upon samples taken at the same time as those for the O_2 combining power. The percentages found are given in the following table.

Percentage of sugar in blood of W. W. P.

	BEFORE MORPHINE		$1\frac{1}{2}$ HOURS AFTER MORPHINE
	<i>per cent</i>		<i>per cent</i>
Sample 1.....	0.15	Sample 4.....	0.14
Sample 2.....	0.11	Sample 5.....	0.14
Sample 3.....	0.11	Sample 6.....	0.12
Average.....	0.12	Average.....	0.13

The micromethod of Bang (49) was used, and the results obtained are so near to each other that one can conclude that there was no appreciable increase in the blood sugar. Calculations of the amount of change of fat to sugar necessary to lower the quotient from 0.75 to 0.70 for 2 hours as occurred with W. W. P. show that about 7 grams of glucose as a maximum might have been formed and whether this is sufficient to change the percentage of sugar in the blood perceptibly is a matter of doubt; thus one hesitates to conclude that such a change did not occur merely because no appreciable rise in the blood sugar was found. The often noticed occurrence of glycosuria and hyperglycemia in morphine poisoning (especially in cats), although generally ascribed (50) to formation of glucose from glycogen in the liver, leads us to think that this possibility of morphine causing a change of fat to carbohydrate is worthy of further study. We have found no literature on the lowered respiratory quotient after morphine. Calculating from figures of Winternitz in one experiment, heroin did not give a fall, but on the contrary a rise in the respiratory quotient as shown in a preceding table.

Comparing heroin and morphine, one finds in our experiments but few indications of differences. The alveolar CO_2 tension, pulse, respiration rate, and gaseous metabolism seem to be affected about the same by both drugs. The "dead space" became smaller (broncho-constriction) in both subjects with heroin, but with only one after morphine.

In conclusion, one may say that in these experiments with normal individuals, morphine and heroin in therapeutic doses acted as respiratory depressants, sometimes by acting directly on the center and sometimes by broncho-constriction and in one instance by both. The total gaseous metabolism showed as a rule no change in oxygen consumption (or a very slight diminution) and a marked drop in the carbon dioxide elimination; whether this is due to metabolic changes or to storage of CO_2 due to the respiratory depression is not clear. The respiration rate usually showed a small increase and the pulse rate a small decrease.

Morphine

TIME	ALVEOLAR CO ₂ TENS. <i>mm.</i>	CO ₂ PRODUCED PER MIN. <i>cc.</i>	O ₂ ABSORBED PER MIN. <i>cc.</i>	R. Q.	PULSE RATE	RESPIRATION RATE	VOLUME EX- PIRED AIR		DEAD SPACE <i>cc.</i>
							Per min. <i>liters</i>	Per resp. <i>cc.</i>	

Subject H. L. H. June 6, 1914

9.04 a.m.	Lay on couch, to begin quiet period; did not sit up until end of experiment.								
9.10	38.6								
9.20	40.2								
9.35-9.50		190	238	0.795	73	11.9	4.74	399	102
9.52	39.6								
9.54-10.07		177	230	0.770	67	12.7	4.62	364	106
10.09	39.9								
10.12	16 milligrams morphine sulphate injected left arm.								
10.17	38.8								
10.22-10.37		168	234	0.715	69	12.4	4.36	352	104
10.39	41.2								
10.52-11.06		172	232	0.740	71	12.3	4.45	363	120
11.08	42.4								
11.20-11.35		178	232	0.770	68	12.2	4.42	362	108
11.39	41.8								
11.48-12.00		178	232	0.765	69	13.1	4.48	341	110
12.03 p.m.	43.8								
12.14	0.32 grams caffeine sodium benzoate injected left arm.								
12.19-12.33		200	238	0.840	67	11.9	4.96	417	125
12.35	41.9								
12.55	41.7								
12.59-1.10		189	246	0.770	67	13.2	5.04	382	130
1.13	40.4								

Subject J. H. M. June 5, 1914

8.15 a.m.	Lay on couch, to begin quiet period; did not sit up until end of experiment.								
8.45	40.9								
8.48-8.59		223	305	0.730	64	12.7			
9.04	41.2								
9.12-9.23		210	257	0.820	59	13.8			
9.30	41.7								
9.40-9.51		200	243	0.820	59	13.6	5.66	416	150
10.20	40.5								
10.36	16 milligrams in morphine sulphate injected left arm.								
10.43	40.9								
10.45-10.56		190	275	0.690	64	12.7	5.15	406	137
11.02	42.2								
11.08-11.19		180	286	0.630	59	14.3	5.12	358	129
11.25	41.2								
11.45-11.56		179	265	0.675	54	15.3	5.40	353	140
12.01 p.m.	41.8								
12.20-12.31		169	263	0.645	52	15.0	5.10	340	135
12.37	42.0								
1.10-1.21		183	246	0.740	47	13.7	5.20	380	139

Morphine—continued

TIME	ALVEOLAR CO ₂ TENS. mm.	CO ₂ PRODUCED PER MIN. cc.	O ₂ ABSORBED PER MIN. cc.	R. Q.	PULSE RATE	RESPIRATION RATE	VOLUME EX- PIRED AIR		DEAD SPACE cc.
							Per min. liters	Per resp. cc.	

Subject J. H. M. Nov. 8, 1914

8.45 a.m.	Lay on couch, to begin quiet period; did not sit up until end of experiment.								
9.15	39.5								
9.21-9.32		191	236	0.810	58	12.4	5.18	418	150
9.35	41.0								
9.40-9.51		197	248	0.795	57	12.8	5.26	411	145
9.55	40.2								
9.59-10.09		198	263	0.750	54	12.9	5.30	411	145
10.15	40.7								
10.18	16 milligrams morphine sulphate injected left arm.								
10.30	40.8								
10.34-10.44		179	259	0.690	67	14.6	4.89	335	123
10.55	41.6								
10.56-11.07		170	245	0.695	59	14.0	4.49	321	107
11.15	40.6								
11.19-11.33		172	236	0.730	56	14.3	4.65	325	114
11.35	41.4								
11.50-12.03		160	225	0.710	51	13.3	4.27	321	111
12.10	40.8								
12.17-12.31		159	223	0.715	53	12.8	4.32	338	122

Subject W. W. P. Dec. 22, 1914

8.10 a.m.	Lay on couch, to begin quiet period; did not sit up until end of experiment.								
8.50	35.7								
9.05-9.17		207	279	0.740	67	10.2	4.97	488	77*
9.20	36.2								
9.25	35.4								
9.27-9.39		209	283	0.740	66	10.9	5.14	472	89
9.45	37.1								
9.48	First sample of blood taken from vein.								
9.53-10.05		223	292	0.765	65	11.8	5.42	459	89
10.10	36.9								
10.13	16 milligrams morphine sulphate injected left arm.								
10.25	38.1								
10.29-10.41		205	271	0.755	64	13.8	5.15	373	83
10.45	36.6								
10.51-11.03		201	289	0.695	64	13.7	5.07	370	84
11.06	38.3								
11.11-11.23		193	276	0.700	61	13.8	5.05	366	100
11.35	37.8								
11.40	Second sample of blood taken from vein.								
11.53-12.05		187	287	0.655	59	13.3	4.82	362	94
12.06	38.4								

* Lying on back; other experiments were lying on left side.

Heroin

TIME	ALVEOLAR CO ₂ TENS. mm.	CO ₂ PRODUCED PER MIN. cc.	O ₂ ABSORBED PER MIN. cc.	R. Q.	PULSE RATE	RESPIRATION RATE	VOLUME EX- PIRED AIR		DEAD SPACE cc.
							per min. liters	per resp. cc.	

Subject J. H. M. May 5, 1914

8.50 a.m.	Lay on couch, to begin quiet period; did not sit up until end of experiment.								
9.00	38.8								
9.12-9.28		200	238	0.835	58	13.1	5.47	418	148
9.35	40.3								
9.44-9.56		197	242	0.815	56	13.1	5.33	406	148
10.05	40.3								
10.20	41.5								
10.26	5 milligrams heroin hydrochloride injected left arm.								
10.35	40.9								
10.39-10.56		159	241	0.660	57	13.6	4.16	306	105
10.58	42.8								
11.06-11.20		164	232	0.705	56	13.6	4.22	311	106
11.25	42.1								
11.45	41.6								
11.49-12.06 p.m.		165	227	0.730	50	13.8	4.36	317	110
12.10	41.5								
12.21	0.4 gram caffeine sodium salicylate injected left arm.								
12.25	41.1								
12.27-12.40		178	234	0.760	52	13.3	4.73	356	109
12.45	37.0								
12.48-1.05		180	241	0.745	50	12.0	4.75	396	115
1.08	39.3								

Subject H. L. H. May 29, 1914

8.00 a.m.	Lay on couch, to begin quiet period; did not sit up until end of experiment.								
8.30	38.8								
8.38-8.44		198	238	0.835	75	13.6	6.56	482	129
8.50	38.7								
8.52-9.04		193	226	0.855	72	13.3	6.21	467	110
9.13	38.3								
9.22-9.34		198	237	0.835	72	15.4	6.82	443	121
9.40	39.6								
10.18	5 milligrams heroin hydrochloride injected left arm.								
10.24	38.7								
10.31-10.43		186	237	0.785	72	14.7	6.29	428	112
10.51	40.6								
11.07-11.20		189	241	0.785	71	13.5	5.86	434	95
11.25	40.0								
11.39-11.50		178	246	0.725	71	13.0	5.55	427	92
11.55	40.2								
12.11-12.23		179	229	0.785	65	13.7	5.82	425	99
12.25	38.8								

SUMMARY AND CONCLUSIONS

Observations upon the alveolar CO₂ tension, the respiration rate and ventilation of the lungs, the gaseous exchange together with calculations of the size of "dead space" in breathing in normal human subjects, following the administration of the usual therapeutic doses of atropine, caffeine, camphor, strychnine, morphine and heroin, are reported and the various actions found with these drugs are summarized in the form of the following table.

DRUG	AVERAGE DOSE	ACTION				
		Respiratory center	Bronchial musculature	Metabolism	Respiration rate	Pulse rate
Atropine.....	1.0 mg.	none	dilation	increase	none	fall then rise
Caffeine.....	0.4 gram	stimulation	either dilation or none	increase	increase	none
Camphor.....	0.1 gram	none	either dilation or none	generally slightly increased	none	none
Strychnine...	4.5 mg.	none	Probably none	none	none	none
Morphine.....	16.0 mg.	either depression or none	constriction*	either slight decrease or none	slight increase†	none or decrease
Heroin.....	5.0 mg.	depression	constriction	none	none	slight decrease

* Or none, when the bronchi are already constricted.

† This obviously does not apply to large doses of morphine.

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INTRAPERICARDIAL MEDICATION AND MASSAGE IN THE TREATMENT OF ARREST OF THE HEART

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Many attempts have been made to discover a reliable method of starting an arrested heart, some method which would be of practical utility not only for experimental mammals but also for man. It need hardly be said that the subject is of importance, because in certain cases of death, while it is true that arrest of the heart is tantamount to absolute loss of the power of spontaneous recovery, yet death is incidental in the sense (1) that the heart may not be of itself irremediably beyond the power of recovery, and (2) that, given recovery of the heart, the other organs may also be capable of continuing their functions.

Three methods of starting an arrested heart have chiefly been employed: (1) by massage; (2) by drugs; (3) by electrical stimulation. Only the former two methods will be considered here.

With regard to the possibility of starting the heart by means of drugs, the methods hitherto employed have been of little practical value, not so much on account of the inefficacy of the drugs employed as from the difficulty of getting those drugs into intimate contact with the whole heart. When the circulation is totally arrested, this difficulty is inevitable. Even intravenous injection into a vein as near the heart as possible probably reaches little more than the right auricle, and certainly until the heart gives at least one beat it is impossible in this way to reach the whole heart muscle. A point which is sometimes overlooked is that, until the heart begins to beat, intravenous injection can at most reach only the interior of the chambers of the right side of the heart. It is only after the blood containing the drug injected has passed through the pulmonary circulation

and through the left side of the heart that it can get into the aorta, and so, by the coronary vessels, into the substance of the heart muscle. In other words the blood supply of the heart muscle of mammals is not derived immediately through the endocardial surface but through the coronary vessels, and drugs must reach these vessels before they can exert anything more than a localised action on the heart.

Partly owing to the limitations of intravenous injection in arrest of the circulation, it has been suggested and attempted to resuscitate the arrested heart by means of drugs injected directly into the muscle or the interior of the heart. This again gives only a localised action on one part of the heart; and, while injection into an auricle is technically difficult and also risky on account of haemorrhage, injection into a ventricle brings the drug injected into contact with the part of the heart which is not normally responsible for originating the beat, and which has the feeblest power of spontaneous rhythm.

There is, however, a third possible method of getting drugs to an arrested heart, which, so far as we can find, has not been suggested and in any case has not come into use, and that is by injection into the pericardial sac. It appeared that by this procedure combined with massage, a solution of a drug could be brought into contact with the exterior of the whole heart. That this method has not been investigated is all the more remarkable in that, prior to the advent of perfusion methods, the action of drugs on the frog's heart was determined chiefly by application to the heart's surface.

The value of this method, however, must depend upon how far a drug in solution in the pericardial sac can act in mammals on the heart muscle through the visceral epicardium. It is of course well known that drugs produce their actions when applied to the surface of the frog's heart, though it has been found that, when applied in this way, they may have a somewhat different effect from that which they produce when perfused through the heart, and also that, to produce the same effects, they must be employed usually in higher concentrations.

In order to estimate the value of intrapericardial injections combined with massage, it was necessary in the first place to obtain some idea of the effect of each of these procedures separately. The main object of this inquiry was, therefore, directed towards obtaining some data which might be of practical value as a basis for the treatment of an arrested heart, but for convenience, our experiments may be considered as attempts to determine the following points: (1) the extent to which certain drugs exert their actions when introduced into the pericardial sac; (2) the best method of performing massage of the heart; (3) the value of intrapericardial injections combined with massage in the treatment of an arrested heart.

I. Action on the heart of drugs introduced into the pericardial sac

For our purpose it was of course essential to determine whether drugs can act on the heart through the visceral epicardium, and if so whether they act quickly. An action obtained by absorption into the general circulation from the pericardium would obviously offer no advantages, in the treatment of arrest of the circulation, over an action obtained from intraperitoneal or intravenous injection.

It was also necessary to select drugs which can act quickly and whose effects are readily demonstrable. We therefore made preliminary experiments with atropine, pilocarpine and adrenaline which fulfil these conditions.

Methods. We shall deal later with the method of injecting solutions into the pericardial sac by means of a needle inserted through the intact thoracic wall; in preliminary experiments it was desirable to insure that the solution actually reached the sac. For this reason and also to permit immediate observation of the heart, and, in later experiments, to allow massage of the heart, an opening was made in the thoracic wall.

The general procedure was as follows. The animals (rabbits) were anaesthetised by urethane (2.5 to 3 grams per kilo subcutaneously). A cannula was then inserted into the trachea, through which, as desired, natural respiration could proceed, or artificial respiration could be applied, with or without a volatile

anaesthetic, by means of Brodie's arrangement. The left carotid artery was connected with a manometer to record the heart beats and blood-pressure, and a cannula was inserted into the right external jugular vein to allow intravenous injections if desired. The opening in the thoracic wall was made on the right side and with the following precautions to prevent haemorrhage. By means of a blunt aneurism needle, a ligature was tied round each of three adjacent ribs in two places—close to the sternum and nearer the vertebrae. The part of the middle rib between its two ligatures could then be resected without haemorrhage. Through the opening solutions were injected by a fine hypodermic needle into the pericardial sac, and through such an opening direct massage of the heart could be performed in later experiments.

Artificial respiration was of course necessary immediately before, and continuously after, the thorax was opened.

Experiment 1. Shielded electrodes were applied to the right vagus nerve. Faradic stimulation of it with the secondary coil at 140 mm. was found regularly to produce arrest or marked inhibition of the heart.

Atropine sulphate, 0.4 cc. of a one per cent solution, was injected into the pericardial sac. Thirty seconds later, stimulation at 140 produced slight slowing, and eighty seconds after injection, was without effect. Two minutes after injection, stimulation at 100 produced no effect on the heart, and three minutes later no slowing was produced by stimulation with the coil at zero.

The result was not due to local injury of the vagus by repeated stimulation, because when the electrodes were subsequently applied to the left vagus, the result was the same.

An opening having been made in the abdominal wall to permit observation of the intestines, 0.5 cc. of a one per cent solution of pilocarpine nitrate was injected intravenously. This dose is sufficient in a normal animal to cause marked and prolonged slowing or even temporary arrest of the heart. In this case, however, it produced merely slight slowing for two or three beats, but caused the usual marked increase of the intestinal movement.

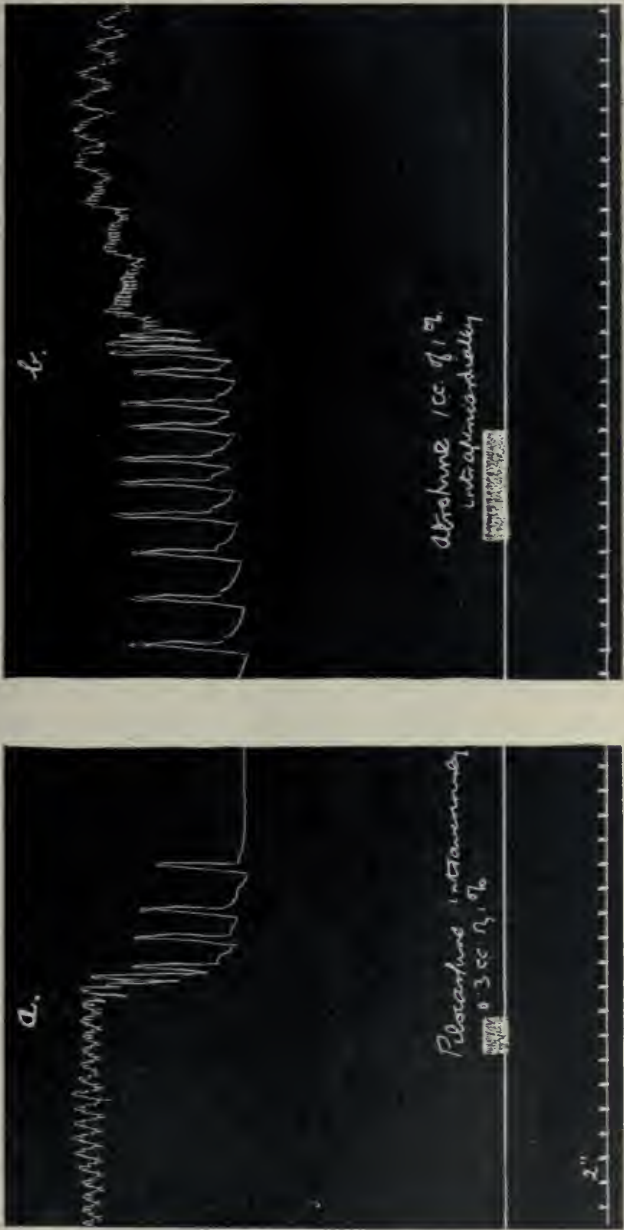


FIG. 1. RABBIT. URETHANE
Showing (a) slowing of heart by intravenous injection of pilocarpine; (b) prompt quickening of heart by subsequent intrapericardial injection of atropine.

This experiment shows that atropine injected into the pericardial sac rapidly paralyses the terminations of the vagus in the heart, with sufficient completeness to nullify the effect on the heart not only of vagus stimulation but of intravenous injection of pilocarpine. The fact that pilocarpine continued to produce its usual effect on the intestines rendered it probable that the action of atropine was a local one upon the heart through the visceral epicardium, and not due to absorption of it into the general circulation.

Experiment 2, Figure 1. The heart rate before injection was 40 per ten seconds. Pilocarpine nitrate, 0.3 cc. of a one per cent solution, was injected intravenously (fig. 1 a). This produced a transient arrest of the heart, after which beats were resumed at the rate of about 4 per ten seconds. Atropine sulphate, 1 cc. of a one per cent solution, was then injected into the pericardial sac (fig. 1 b), and twenty seconds later the heart-rate had increased to 35 per ten seconds, and the blood-pressure was practically at its normal level.

This experiment again shows the rapidity with which atropine acts on the heart when introduced into the pericardium.

Experiment 3, Figure 2. This experiment was made to determine to what extent pilocarpine produces cardiac slowing when injected into the pericardium.

The heart rate before injection was 20 per four seconds (fig. 2 a). Pilocarpine nitrate, 0.5 of a one per cent solution, was injected into the pericardial sac. In twenty seconds the heart rate was 14 (b), in one minute 12 (c), and in three minutes 8 (d), per four seconds. The slowing was a genuine action of pilocarpine, because intravenous injection of atropine (d) produced immediate acceleration of the heart, restoring it in two minutes to its original rate of 20 per four seconds (e).

That the slowing produced by pilocarpine in this experiment was due to a local action on the heart and not to absorption into the general circulation, was presumed from the fact that twice as big a dose of pilocarpine injected into the peritoneal cavity in a control animal under similar conditions reduced the heart rate in five minutes only from 40 to 37 per ten seconds.

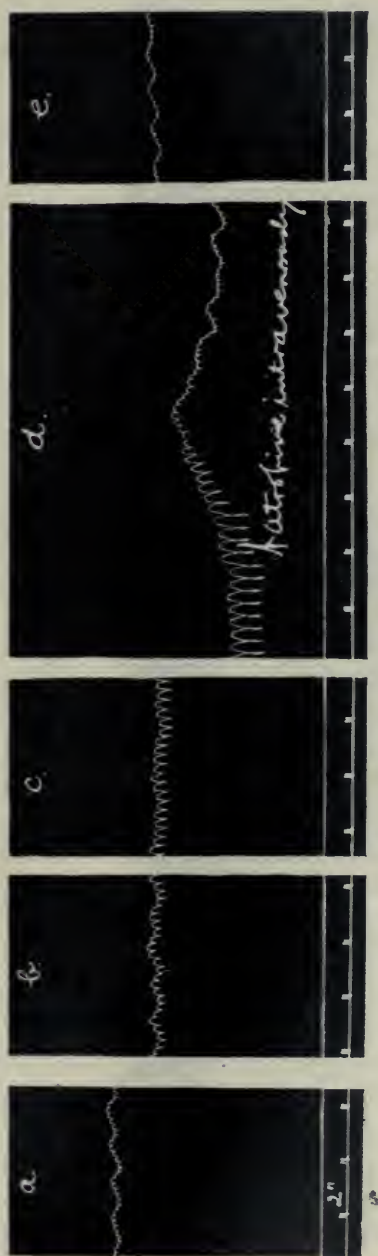


FIG. 2. RABBIT. URETHANE

Showing (a) normal heart rate. Between (a) and (b) pilocarpine nitrate was injected into the pericardial sac. This produced gradual slowing of the heart (b), (c), and (d). The slowing was quickly removed by intravenous injection of atropine (b) and (e).

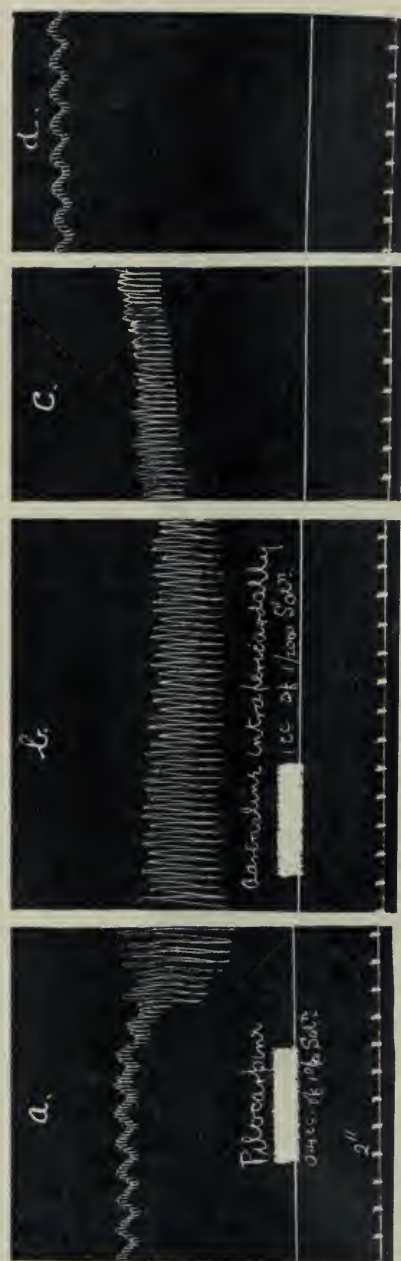


FIG. 3. RABBIT. URETHANE

Showing (a) slowing of heart by intravenous injection of pilocarpine; acceleration of heart by subsequent intrapericardial injection of adrenaline (b), (c), and (d).

Experiment 4, Figure 3. This experiment was made to determine whether adrenine acts on the heart when introduced into the pericardial sac.

The heart-rate before injection was 39 per ten seconds. Pilocarpine nitrate (0.4 cc. of a one per cent solution) was injected intravenously (fig. 3 a). In half a minute this had reduced the heart-rate to 15 per ten seconds, and adrenine (1 cc. of a 1 in 2000 solution) was injected into the pericardial sac (b). In thirty seconds the heart-rate had increased to 22 (c), and in two minutes to 37, (d), per ten seconds.

Adrenine here produced a definite acceleration of the heart when applied to its external surface. The fact that the blood-pressure rose to above the normal suggests that some adrenine may have been absorbed into the general circulation, though it may on the other hand have been due to an augmentor action on the heart itself.

Experiment 5, Figure 4. This experiment was performed on a cat, the conditions of experiment being the same as in previous experiments. In the more slowly beating heart of the cat, it is easy to show the accelerating action of adrenine without previous use of pilocarpine.

For five minutes before injection the heart-rate had remained regularly 23 or 24 per ten seconds. Adrenine (1 cc. of a 1 in 10,000 solution) was injected into the pericardial sac. In thirty seconds this increased the heart-rate to 32 per ten seconds (fig. 4). The effect of adrenine in this experiment is clearly due to a local action on the heart, the acceleration being very considerable but the rise of blood-pressure only very slight.

These and other experiments have furnished sufficient grounds for believing that at all events atropine, pilocarpine and adrenine can exert their actions on the heart when introduced into the pericardial sac. In regard to the present inquiry adrenine was the most interesting; one of us has in recent years conducted a series of experiments to determine which is the most powerful cardiac stimulant from the point of view of its value in starting an arrested heart; and, of those drugs which have been tried, adrenine has proved unquestionably the best. Thus it has been

shown (Quart. Journ. Exper. Phys., vii, p. 75, 1913) that adrenine can antagonise concentrations of chloroform which considerably depress the heart and concentrations of chloral which even arrest it. The object of this investigation would have been achieved, and the question of proof simplified, if it had been found possible to arrest the heart by chloroform or chloral and to start it again by intrapericardial injection of adrenine, without further complication. We have performed many experi-

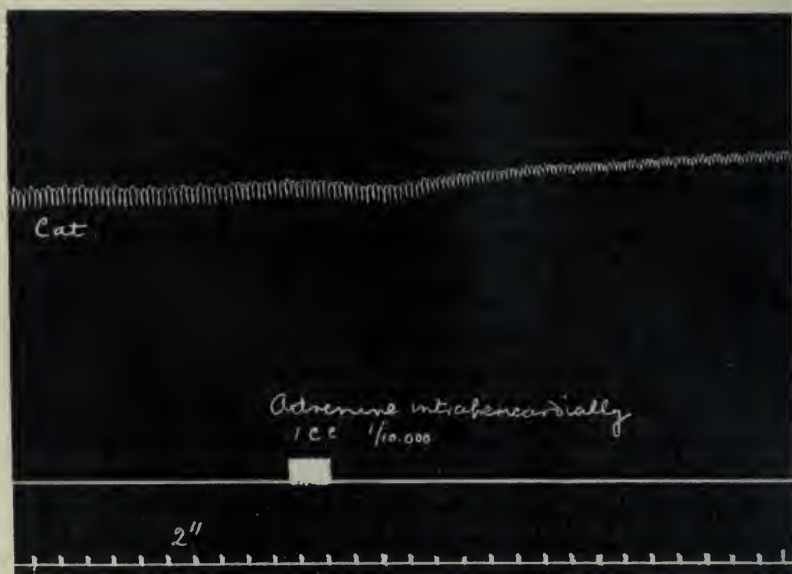


FIG. 4. CAT. URETHANE AND CHLOROFORM
Showing acceleration of heart by intrapericardial injection of adrenine.

ments to determine whether this were possible, without being successful in any instance. At its best, intrapericardial injection can hardly be comparable to perfusion through the coronary vessels, so that this negative result was not contrary to expectation. Indeed the fact that the heart will continue to beat after the injection of 1 in 1000 solution of adrenine into the pericardium, shows that this drug produces only a limited action in this way. Possibly its absorption is self-limited by contraction of the arterioles. However, we think that the slight stimulant

action of adrenine obtainable by this method of administration is not negligible, but is of definite value in starting an arrested heart, when combined with massage. It is, moreover, difficult without massage to spread an intrapericardial injection of small bulk over the whole heart. Once the complication of massage is introduced, then it becomes necessary to determine the effect of massage per se, otherwise it is difficult to know what value to attach to the superadded intrapericardial injection. This led us to attempt to standardise a method of cardiac massage, and, so far incidentally, to determine the best method of performing it.

II. Massage of the heart¹

Since its introduction by Schiff as a method of reviving the heart, rhythmic compression of the heart has been employed often in experimental mammals and occasionally in human beings. There can be no doubt in regard to its value; and, when combined with intrapericardial injections of adrenine, it has succeeded in our hands, for example, in reviving the rabbit's heart an hour after it had been arrested by chloroform.

So far as we can find, previous observers have been content to use the expression "massage of the heart" as if the manner of it were indifferent. On the contrary, we believe, as the result of a large number of experiments, that attention to certain points of detail may make all the difference between success and failure in difficult cases of resuscitation of the arrested heart. Moreover, as the time which the central nervous system of mammals can survive complete anaemia without permanent injury to the brain cells has been calculated by Neil Stewart and others to be something short of fifteen minutes, it is obvious that any method which is to be used in man to revive an arrested heart must admit of starting the circulation within that time. It is clear therefore that no detail is too insignificant for attention in regard to massage, if it is to be employed in man, because any modifica-

¹ A full account of the literature on resuscitation of the mammalian heart has recently been given by Pike, Guthrie and Stewart, *Journal of Experimental Medicine*, x, p. 381, 1908.

tion in the manner of performing it which shortens, even by a few minutes, the arrest of the circulation will make all the difference in the justification of its employment.

Partly for this reason, and partly because results arrived at empirically appear to acquire a perfectly rational explanation provided one has a sufficiently clear conception of what cardiac massage is really meant to accomplish, we propose to consider the question in some detail at the risk of mentioning some points which may appear elementary.

For convenience we may take as an example a heart which has been arrested by an overdose of chloroform, and assume that the effect is produced by accumulation in the blood of a concentration of chloroform sufficiently great to paralyse the heart muscle. With obvious modifications, the same argument is applicable to a heart arrested by asphyxia, only that in the latter case resuscitation is easier.

Artificial respiration is of course necessary. In chloroform-poisoning this by itself will never in our experience start the heart beating, if the circulation has been completely arrested. The question is, therefore, what does cardiac massage do which enables it to start the heart when added to artificial respiration.

Rhythmic compression of the heart effects in the first place an artificial circulation; it produces movement of blood in the vessels to an extent which depends on the way in which it is done. As a gauge of the mechanical efficiency of massage of the heart, we have used the effect produced upon the manometer connected with a carotid artery, as in ordinary blood-pressure experiments. The records are therefore subject to the same limitations as records of blood pressure, but are less complicated in so far as the peripheral resistance may be regarded as constant, and the compressions as beginning each time practically at the zero of blood-pressure. The amplitude of the wave recorded depends in this case sufficiently nearly upon the amount of blood propelled into the aorta by each compression of the heart. The slower the rhythmic compression (if the force of it be constant) the larger will be the pulsations of the manometer, provided that the longer pause ensures a more complete filling of the heart.

In the first place we have endeavoured to ascertain to what extent the mechanical efficiency of massage depends on the method employed. In the experimental mammal, massage may be attempted in four chief ways: (1) by simple compression of the thorax; (2) by direct compression of the heart through a window made in the thoracic wall by resection of part of one or more ribs or of the sternum; (3) by simple compression of the abdomen; (4) by compression of the heart from below the diaphragm through an opening in the abdominal wall.

Briefly, we have found that in order of mechanical efficiency those methods arrange themselves as follows: (1) direct compression through an opening in the thorax; (2) compression through an opening in the abdomen; (3) simple abdominal compression; (4) simple thoracic compression. The methods follow that order in regard not only to the amount of blood propelled into the aorta by them, but also to their value in starting an arrested heart.

To take the case of the most effective method—direct compression of the heart through an opening in the thorax—how far does it ensure actual circulation of the blood? Massage was done by compressing the ventricles (by one finger introduced into the opening) against the left thoracic wall.

When the ventricles are compressed, blood is driven into the aorta (of which there was a record) and into the pulmonary artery; the closure of the auriculo-ventricular valves prevents, more or less, the escape of blood into the auricles. When compression is removed the ventricles resume their normal diastolic distention. They draw blood chiefly from the veins *via* the auricles, the aortic and pulmonary valves preventing much reflux from the arteries.

That an actual circulation is effected by massage was shown by the following experiment. A solution of Erioglaurine A was injected into the cavity of the right ventricle of an arrested heart. Cardiac massage was then performed, and after a few compressions, the blue-green colour appeared first in the lungs and then in the carotid artery. Massage, therefore, had propelled the blood from the right ventricle through the lungs and left side of the heart into the systemic circulation.

One point in regard to which we think mistakes have been made is to imagine that the rate of massage should correspond to the normal heart-rate. Reflection and experiment show that this is wrong. In the normally beating heart the ventricles fill by active propulsion of blood from the auricles, and the circulation is aided by other factors which are now in abeyance; in the case of massage of an arrested heart the filling is effected by a suction process due to the elasticity of the ventricles, which takes a longer time. The most effective rate of compression is that which allows almost complete filling of the ventricles, and is much slower than the normal heart-rate. This is so also for another reason. When the heart begins to beat again after arrest, it beats much below the normal rate, and it is this subnormal rate that one is attempting to provoke by massage. The rate of rhythmic compression should be certainly less than half the normal rate.

It follows, moreover, from these considerations that, while compression should be done somewhat gradually to avoid injury to the heart, relaxation should be abrupt and jerky, so as to obtain the maximum amount of suction of blood from the auricles in the shortest time.

The effect of massage and artificial respiration in a heart arrested by chloroform requires further consideration. While artificial respiration is being carried on, the chloroform is being eliminated from the lung capillaries. The first compression of the heart drives chloroform-laden blood into the aorta and pulmonary arteries, draws similar blood into the right auricle, but more or less chloroform-free blood from the lungs into the left auricle. Subsequent compressions move on the chloroform-free blood into the root of the aorta. It must, however, take some considerable time before this is moved on through the whole systemic circulation in this way, and before the concentration of chloroform is conspicuously lowered in the right side of the heart. Nevertheless, under certain circumstances, one or two compressions may start the heart beating after it has been arrested by chloroform, especially if artificial respiration has been carried on for some time before massage is begun. This was at

first a matter for some surprise until it was realized that blood coming from the lungs to the left side of the heart, as soon as it reaches the root of the aorta, will go to the coronary vessels and therefore to the substance of the heart muscle, some time before the chloroform-free blood has been moved on through the whole systemic circulation. The same considerations apply to the conveyance, by massage, of oxygenated blood to the arrested heart.

Judged merely from the point of view of its acting as an artificial circulation, massage would be most efficacious when carried out uninterruptedly, because the maximum amount of circulation of blood would thereby be accomplished, and therefore the speediest elimination of chloroform. However we have repeatedly found that this is not the case, the reason being that this procedure fails to take due cognisance of the second function of massage, that, namely, of acting as a mechanical stimulus to the heart itself. The latter is the real object aimed at in performing massage; and, though a mechanical circulation may be a necessary preliminary to obtaining conditions under which the heart can resume its action, it is clear that, even from the point of view of circulating the blood, cardiac massage is an inadequate substitute for the beating heart. It is essential that this mechanical stimulation should be applied in such a way as most effectively to encourage the spontaneous contractions of the heart. This is not attained by continuous massage. It has been found for example that massage, which has started feeble spontaneous beats, if continued without intermission, may cause the beats to disappear again. No doubt it is possible to overdo mechanical stimulation, and continuous massage does not give the heart a sufficient opportunity of developing spontaneous beating. We have found, as a result of a large number of experiments, that when the feeblest beats have begun, even beats which fail to move the manometer, it is better to cease massage for some seconds so as to allow them to develop, and to apply short periods of massage intermittently, when it is usually found that perhaps by the third or fourth series the heart suddenly regains a normal rhythm and the blood-pressure rises rapidly.

This method of performing intermittent massage is shown in figure 5, which illustrates the end of successful massage of a heart stopped by chloroform and arrested for fifteen minutes. Adrenine had previously been injected into the pericardium. Between the second and third periods the heart was beating very feebly, a movement of the manometer being just detectable. Between 3 and 4 the beats are easily visible, between 4 and 5 the heart was beating with considerable vigour; the fifth short period of massage starts the heart beating normally and the blood-pressure rises rapidly. If the heart had been massaged continuously after 3, it is quite possible that the beats would have disappeared again, and, but for a temporary interruption of massage, the spontaneous contractions would probably have been overlooked.

In some cases, when the circulation has been completely arrested for many minutes, it is found that the heart's contractions can be induced to develop only up to a certain point, and that the blood-pressure remains low. The feebleness of the heart is in part secondary to the low blood-pressure, the vasomotor centre being paralysed. In such cases an intravenous injection (which is now effective with the blood circulating) of either pituitrin or adrenine will often produce immediate and permanent improvement both of the heart and of the blood-pressure.

An example of this is shown in figure 6, (ii). Figure 6 illustrates the complete process of resuscitation of a heart which was revived only with difficulty. Chloroform had been administered until the heart stopped, and resuscitation was begun fifteen minutes later. Artificial respiration was applied and adrenine injected into the pericardial sac (fig. 6 a). After six series of compressions very feeble spontaneous beats of the heart began (b). The tracing shows that the spontaneous beats are slow at first, and, as has previously been emphasised, it is this slow rate which one attempts to elicit by massage. The heart was massaged intermittently until it continued to beat regularly (c). Blood-pressure, however, continued low and showed no tendency to rise further. Three minutes after regular beats had commenced, (d) adrenine (0.1 mgm.) was injected into the marginal vein of



FIG. 5. RABBIT, HEART ARRESTED BY CHLOROFORM

Showing method and results of applying intermittent massage. The periods of massage are marked 1 to 5.

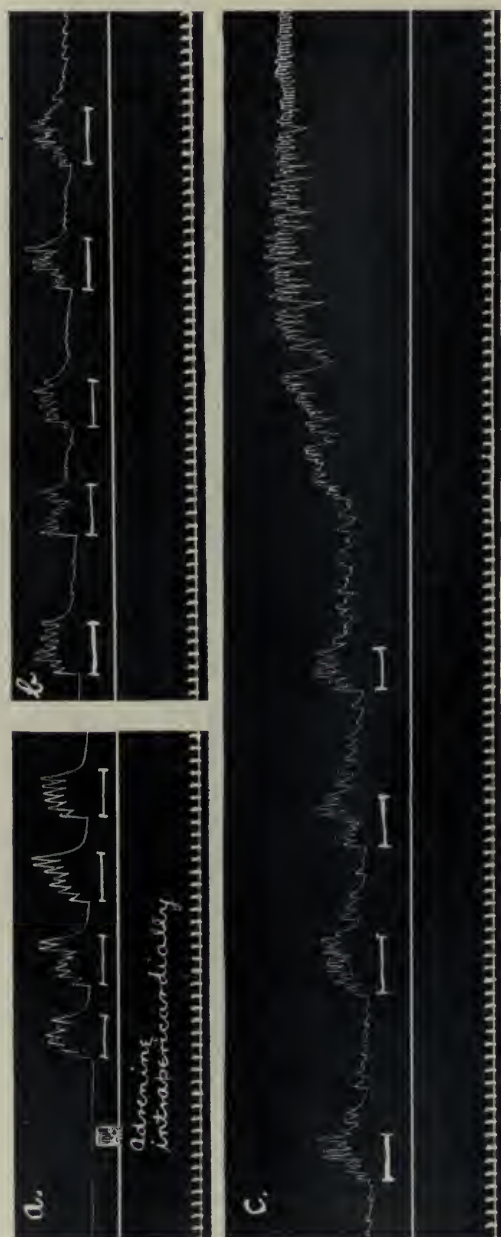


Fig. 6 (i).



Fig. 6 (ii).

FIG. 6. RABBIT. HEART ARRESTED BY CHLOROFORM

Showing complete resuscitation (see text). The periods of massage are marked. By the end of Fig. (b) feeble spontaneous beats had commenced, and by the end of (c) the heart was beating regularly. Blood pressure remained low. Intravenous injection of adrenaline produced an abrupt rise of pressure (d). The pressor effect was maintained (e, taken 20 minutes later).

the ear. This produced an abrupt rise of blood-pressure from 24 mm. to 120 mm., falling in two minutes to 90 mm. A marked improvement in the heart-beats was simultaneously observed (by inspection). Twenty minutes later, the blood-pressure was 84 mm. (e). Adrenine obviously produces a more permanent rise of blood-pressure under these conditions than when injected into an animal with a normal blood-pressure.

III. Intrapericardial injections of adrenine combined with massage in the treatment of arrest of the heart

For reasons to be stated immediately, we have not attempted in the previous section to differentiate between the effects of massage alone and of massage combined with intrapericardial injections of adrenine. In the first section it was shown that adrenine, when introduced into the pericardial sac, exerts a definite local stimulant action on the heart. One of us had previously found² that adrenine, perfused through the heart, can to a certain extent antagonise the depressant action of chloroform, but, as has been already stated, it was not found possible to start a heart arrested by chloroform simply by injecting adrenine into the pericardial sac. Nevertheless there was reason to believe that such stimulant action as could be obtained in this way would be of some value in starting the heart; and this value could be estimated only somewhat indirectly, for the following reasons.

When chloroform is administered to a rabbit by ordinary inhalation until the heart stops, massage, if applied immediately, is frequently by itself sufficient to start the heart again, when combined with artificial respiration. With such a variable normal, it was found impossible to determine what value, if any, to attach to intrapericardial injection of adrenine, and it was therefore necessary to impose more severe conditions. This was obtained in the following way. With the animal anaesthetised by urethane or chloroform and the chest opened, artificial respiration was maintained by Brodie's respiration pump. Chloroform was then turned on to the maximum concentration possible with Brodie's anaesthetisation apparatus. The conditions were the

² Quart. Journ. Exp. Phys., 1913, vii, 75.

same in all the experiments. This concentration of chloroform arrested the heart, the time taken varying somewhat in different animals. As soon as the heart stopped artificial respiration was discontinued, and, after a certain period it was resumed without chloroform, and the heart massaged. The toxic action of chloroform on the heart was in this way allowed full development, (1) because arrest of the heart was independent of asphyxia, and (2) because the heart was left for a time saturated with a toxic concentration of chloroform.

Under these conditions we have never been able to induce spontaneous beats of the heart by massage alone if an interval of five minutes had elapsed since arrest of the heart. On the other hand, we have revived the heart by massage combined with intrapericardial injection of adrenine after an interval of an hour's arrest. We have performed massage, after ten minutes' arrest, for twenty minutes without inducing any spontaneous beats, and then injected adrenine into the pericardium, whereupon vigorous and permanent contractions appeared after the next few compressions. A large number of such experiments appear to have removed the question beyond the possibility of coincidence, and have convinced us that the intrapericardial injection of adrenine is definitely valuable as an adjuvant to massage in the treatment of chloroform-arrest of the heart.

By the same means recovery of the heart has been obtained after arrest by intravenous injection of chloral, and in other cases as it has occurred.

The experiments which we have so far described obviously only supply certain data to form a basis for a line of treatment of cardiac arrest in man. We have dealt chiefly with arrest of the heart produced by chloroform poisoning, not only because this condition is of practical importance in itself, but also because chloroform is a powerful cardiac poison and, therefore, a method of treatment which is successful in this case is *a fortiori* successful in cardiac arrest due to asphyxia or to other poisons less toxic than chloroform to the heart muscle.

The experiments have shown that in the rabbit the heart can be recovered by massage combined with intrapericardial injec-

tion of adrenine in practically any case of chloroform poisoning, even though the treatment be not begun until fifteen minutes or longer after the heart has stopped. In those experiments arrest of the heart was confirmed by direct inspection through the opening in the thoracic wall.

Further experiments were performed in which the attempt was made (1) to produce in the rabbit death from overdose of chloroform under such conditions of administration as it may occur in man and (2) to evolve a practical method of treatment, based on the foregoing experiments, such as might reasonably be employed in chloroform poisoning in the human subject.

The procedure employed was as follows:

The rabbits were anaesthetised by chloroform in a bell-jar. As soon as they were completely anaesthetised, the bell-jar was removed and concentrated chloroform was administered by the open method until the heart stopped. The animal was then left for ten minutes. A tube was then inserted into the trachea through the mouth and larynx for applying artificial respiration. An opening was made in the middle line of the abdomen below the xiphisternum. Artificial respiration was begun, adrenine injected into the pericardium, and the heart massaged through the abdominal opening.

Before giving the results of these experiments, certain preliminary points require consideration. In the first place, we discarded, as a practical method, the performance of massage through a hole in the thoracic wall, though, as has been previously stated, by this way massage may most effectively be employed. Though it has been tried in the human subject, this method is almost certainly too dangerous and takes too long a time to have any claim to adoption.

In the second place, though in our previous experiments arrest of the heart could be ascertained by direct inspection, this was not now possible. We, therefore, took as the point of arrest of the heart the time when the cardiac impulse could no longer be felt by palpation of the thorax and when the pulse had disappeared from the carotid and femoral arteries. It is not possible to say that the heart had absolutely ceased to beat in

every case, but the point is sufficient for our purpose that it was stopped as far as regards the tests which are clinically applied in chloroform poisoning. Moreover, there was in no case any haemorrhage when the abdominal wall was opened, nor could the slightest contraction of the heart be felt by the finger in the abdomen when massage was begun. It is extraordinary how feeble beats of the heart can be felt by palpation of the thorax in the rabbit, a fact which we have often verified when the thorax was open. We have reason to believe, therefore, that what we took to be cardiac arrest in these experiments would be regarded as cardiac arrest in man. Respiration usually ceased about two minutes before arrest of the heart; sometimes the interval was less, more rarely it extended to ten minutes.

In the third place, in former experiments injections into the exposed pericardium were made directly through the opening in the thorax. It is, however, not a matter of great difficulty even in the rabbit to reach the pericardial sac by inserting a needle through an intercostal space. Preliminary injections had to be made with the open thorax, and then experiments with the unopened thorax in which the position of the injection was subsequently verified by opening the thorax. The pericardial sac can be reached in the rabbit in the following way. A fine hypodermic needle is inserted through the skin, etc., to a depth of 2 cm., entering the fourth left intercostal space about 1 cm. from the mid-sternal line and directed upwards at an angle of about 45° to that line. The amount of adrenine injected was 0.3 cc. of a 1 in 10,000 solution.

Lastly, the opening in the abdomen was made just large enough to admit one finger easily. The heart was massaged through the diaphragm by compressing it against the left thoracic wall.

With experience the insertion of the cannula into the trachea, the intrapericardial injection, and the opening of the abdomen can be done very rapidly. We allowed ten minutes between the arrest of the heart and the commencement of treatment, because no longer time need be required for such a procedure if adopted in man, provided the requisite apparatus be readily available.

The results of experiments under these conditions were that we were successful in recovering the heart in about 70 per cent of cases. In nearly all the remaining cases the heart could be started by direct massage through the opened thorax, showing that the heart was not irrecoverable but that massage by the abdominal route was less effective. One of us hopes, in a future communication, to deal with the question of recovery of tissues other than the heart.

In suggesting this as a possible line of treatment of cardiac arrest in man, either in chloroform poisoning or in certain other conditions, we are of course aware that experiments on the rabbit cannot be directly translated to man. Fibrillation, for example, does not occur readily or completely in the rabbit's heart. We have, however, by the same method recovered the cat's heart, which fibrillates readily, half an hour after arrest by chloroform poisoning. Against this lack of complete parallelism may be placed the fact that, when the human heart has stopped beyond the power of spontaneous recovery, there can be no further risk; and as far as can be judged from our experiments, the suggested method of treatment offers a better prospect of success than any which has hitherto been tried.

SUMMARY

1. Atropine, pilocarpine and adrenaline, when injected into the pericardial sac in the rabbit can rapidly produce their characteristic effects on the heart up to a certain point. The immediate action obtained in this way is a local action on the heart and not simply due to absorption of the drugs into the general circulation.

2. In performing massage of the arrested heart with a view to reviving the spontaneous contractions, the following points are of importance: Compression should be gradual and relaxation abrupt. The rate of compression should be at most less than half the normal heart-rate. Massage should be regularly interrupted at short intervals for a few seconds to allow the spontaneous beats to develop. When the heart is arrested, massage, properly performed, acts both as an artificial circulation and as a mechanical stimulus to the heart muscle.

Massage can be performed in the following ways, arranged in order of efficacy: (1) by direct compression of the heart through an opening in the thoracic wall; (2) by compression of the heart above the diaphragm through an opening in the abdominal wall; (3) by simple compression of the abdomen; (4) by simple compression of the thorax.

3. Direct thoracic massage combined with intrapericardial injection of adrenine can revive the heart in practically any case of chloroform-poisoning in the rabbit, even though the treatment be not begun until fifteen minutes or more after the heart has completely ceased to beat.

In about 70 per cent of cases recovery can be obtained if treatment is commenced ten minutes after arrest of the heart by the following procedure: artificial respiration through a tube in the trachea; intrapericardial injection of adrenine; massage through an opening in the abdomen. If, when the heart has been started, the blood pressure continues low, and the heart beats remain (chiefly as a consequence of this) feeble, speedy and permanent improvement of the heart and blood-pressure can be obtained by intravenous injection of either adrenine or pituitrin.

Treatment on these lines is suggested for cardiac arrest in the human subject, occurring either in chloroform-poisoning or in other conditions in which general recovery might be anticipated to result from resuscitation of the heart.

OBSERVATIONS ON THE EFFECT OF INTRA- VENOUS INJECTIONS OF SODIUM PHOSPHATE

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In a recent publication by E. Starkenstein,¹ entitled "Über die pharmakologische Wirkung kalziumfällender Säuren und der Magnesiumsalze," the claim is made that orthophosphoric, metaphosphoric, pyrophosphoric, phytic (inositephosphoric) and citric acids when administered, in the form of their sodium salts, to animals, whether by mouth, subcutaneously or, preferably, intravenously, produce a fairly consistent complex of symptoms, including muscle-twitching, tremor, convulsions, etc. This effect is explained as being due to their common action in precipitating calcium ions. The administration of calcium salts enabled animals to withstand doses of salts of these acids that would otherwise have been fatal and relieved the symptoms in those in which they had already developed.

These statements were of great interest, particularly in view of the result of chemical analyses of the blood of parathyroidectomized dogs. According to MacCallum and Voegtlin² and MacCallum and Vogel³ such blood contains less calcium than does the blood of normal animals and these authors have ascribed the appearance of tetany after parathyroidectomy to the impoverishment of the tissues in calcium. Moreover, the author⁴ has found that, after parathyroidectomy, there is an

¹ Starkenstein: Archiv für experimentelle Pathologie und Pharmakologie, 77, 45, 1914.

² MacCallum and Voegtlin: Journal of Experimental Medicine, 11, 118, 1909.

³ MacCallum and Vogel: Journal of Experimental Medicine, 18, 618, 1913.

⁴ Greenwald: Journal of Biological Chemistry, 14, 369, 1913.

increase in the phosphorus content of the blood and that this increase is due almost entirely, if not completely, to an increase in the amount of a form of phosphorus closely resembling, and probably identical with phosphoric acid. Particularly noteworthy was the fact that the amount of orthophosphoric acid, per kilo of rabbit, which Starkenstein reported as producing muscle-tremor agreed with the increase in the phosphorus content of the serum of dogs after parathyroidectomy. Thus in experiment 76 (p. 49), the gradual injection of 27 cc. $\frac{N}{3}$ trisodium phosphate solution into a rabbit weighing 1755 grams produced general muscular tremor and 43 cc. produced convulsions. The 27 cc. of solution contained 54 mgm. phosphorus, or about 30 mgm. per kilo of rabbit, an amount frequently exceeded by the increase in the amount of phosphorus in the blood and serum of parathyroidectomized dogs, even before tetany had fully developed. If the observations of Starkenstein could be confirmed they would seem to throw considerable light upon the mechanism of the origin of tetany of parathyroidectomized dogs.

Accordingly, the experiments were repeated on dogs and rabbits. Four solutions were used. These were fifth- and fifteenth-molar solutions of trisodium phosphate and also of a mixture of nine parts disodium and one part monosodium phosphate. The results may be briefly summarized as entirely negative. Very large amounts of these solutions were injected intravenously into the animals without apparent ill effect. Excessively large doses were fatal but no tremor or convulsions were observed. The trisodium phosphate appeared to be slightly the more toxic but the effect seemed to be due to the probable interference with the respiratory exchange caused by the introduction of so much alkaline solution.

These negative results are in complete agreement with the older observations of Falck⁵ and of Gamgee, Priestly and Larmuth.⁶ Falck made three experiments upon dogs. He injected

⁵ Falck: Virchow's Archiv, 54, 173, 1872.

⁶ Gamgee, Priestly and Larmuth: Journal of Anatomy and Physiology, 11, 255, 1877.

intravenously about 200 mgm. phosphorus in the form of tri-sodium phosphate, per kilo body weight, without the appearance of twitching or tremor, save in one experiment, in which a transient spasm occurred. Gamgee, Priestly and Larmuth reported that orthophosphoric acid is practically non-toxic. They injected over 100 mgm. of phosphorus per kilo of rabbit and observed no effect upon the blood-pressure, pulse-rate or behavior of the animal.

In the introduction to the paper under discussion, Starkenstein refers to a previous publication⁷ in these words (p. 46):

"In erster Linie kamen hier für uns die verschiedenen Phosphorsäuren in Betracht, über deren Wirkungsweise ich schon früher untersuchungen angestellt habe, die aber zu keinem endgültigen Resultat geführt haben." On turning to the paper referred to, we find the following (p. 257): "Bei intravenöser Injektion des Tetranatriumsalzes der Pyrophosphorsäure sowie des Tri- und zum teil des Dinatriumphosphats kommt in erster Linie eine Alkaliwirkung in Betracht. Es liegen ähnliche Verhältnisse vor, wie bei der intravenösen Injektion von Seifenlösungen. Eine scharfe Grenze zwischen Salz- und Alkaliwirkung lässt sich aber weder hier noch dort ziehen. . . . Bei subcutaner Injektion zeigen erst unverhältnismässig grössere Dosen der Phosphate eine Giftwirkung, wobei die Salzwirkung in erster Linie mit in Frage kommt. Eine spezifische Wirkung des Säureanions liess sich bei keiner Art der Injektion erkennen." And (p. 259): "So weit überhaupt von einer Giftwirkung der phosphorsauren Salze die Rede sein kann, betrifft diese stats die Kationen." As regards the action of calcium salts it may be well to compare this statement from the later paper (p. 46): "Wie die später mitgeteilten Versuche mit inositolphosphorsaurem Natrium und Trinatriumorthophosphat zeigen, gelingt es, durch subcutane Injektion von Kalziumchlorid auf der Höhe der Vergiftung die tiere zu retten sowie auch durch vorherige Injektion des Ca-salzes den Eintritt der Vergiftungserscheinungen überhaupt zu verhindern, . . . " with this from the earlier one (p. 259): "Es bestand weiter die Möglichkeit, dass die beobachtete Giftwirkung der Phosphosauerstoffverbindungen durch Kalkentziehung hervorgerufen wird. Es wurden daher die geschilderten Versuche an Kaninchen wiederholt, denen vorher grössere Mengen von

⁷ Starkenstein: Biochemische Zeitschrift, 32, 243, 1911.

Calciumchlorid injiziert worden waren. Das Resultat der Versuche erfuhr dadurch keine änderung, so dass dieser erklärungsmodus entfällt."

In short, in his first paper Starkenstein was in complete accord with other observers in this field while in the second he reports experiments, the results of which disagree with all others in the literature, including his own. No explanation of the divergence is given.

EXPERIMENTAL

The injections were made into the femoral vein, under cocaine anesthesia. Before the injection was begun, and at intervals thereafter, a small sample of blood was drawn from the femoral artery of the other side. This was allowed to clot, the serum poured off and centrifugated. In the clear serum, the phosphorus not precipitated by a mixture of picric and acetic acids was estimated by the method elsewhere described by the author.⁸ Only a few of the experiments are here reported. The others differed from these in no important particular.

Experiment 2. Rabbit, female, weight 2.27 kilos. Used a mixture of nine parts of $\frac{M}{5}$ Na_2HPO_4 and one part $\frac{M}{5}$ NaH_2PO_4 .

<i>Time</i>	<i>Injected cc.</i>	<i>"Acid-soluble" phosphorus in 1 cc. serum mgm.</i>
10.00 a.m.		0.0731
10.00 to 10.16	18	
10.16 to 10.21	12	
10.24		0.3955
10.24 to 10.33	50	
10.35		1.049
10.36 to 10.43	48	
10.45		1.432
10.48 to 11.05	80 (approx.)	
11.05		1.514

No twitching or tremor of muscles at any time.

⁸ Greenwald: Journal of Biological Chemistry, 21, 29, 1915.

Experiment 3. Dog, male, weight 12.70 kilos. Used $\frac{M}{15}$ Na_3PO_4 .

<i>Time</i>	<i>Injected cc.</i>	<i>"Acid-soluble" phosphorus in 1 cc. serum mgm</i>
2.07		0.0916
2.07 to 2.15	33	
2.15 to 2.22	50	
2.23 to 2.29	50	
2.30		0.266
2.31 to 2.38	150	
2.39		0.299
2.40 to 2.56	190	
3.05		0.384

After 2.45, the dog was cyanotic but at no time was there any tremor, etc.

Experiment 5. Rabbit, male, weight 2.45 kilos. The serum obtained at the beginning of the experiment contained 0.045 mgm. "acid soluble" phosphorus in 1 cc.

<i>Time</i>	<i>Injection</i>
4.10 to 4.12	25 cc $\frac{M}{15}$ Na_3PO_4
4.20 to 4.32	72 cc. $\frac{M}{15}$ Na_3PO_4
4.32 to 4.35	28 cc. $\frac{M}{15}$ Na_3PO_4
4.37	Animal gasped and died. No tremor was observed at any time.

Blood was obtained from the vena cava and allowed to clot. The serum contained 0.689 mgm. "acid-soluble" phosphorus in 1 cc.

ADDENDUM

Since the above was written, the author has found that Gardner and Symes,⁹ in the course of their work on sodium camphenephosphonates, also administered disodium orthophosphate to cats, without observing any toxic symptoms.

⁹Gardner and Symes, Biochemical Journal, 5, 390, 1911.

ON THE ACTION OF VERATRUM VIRIDE

WITH SOME REMARKS ON THE INTERRELATIONSHIP OF THE MEDULLARY CENTRES

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The use of veratrum viride has recently been strongly recommended by Dr. Haultain¹ in the treatment of eclampsia. According to him the drug is most efficient in reducing a high blood pressure, slowing the pulse and inducing diuresis, and it was at his request that I undertook the following investigation with the object of finding, if possible, the physiological basis for its therapeutic application.

A preliminary survey of the statements made in standard textbooks on the pharmacological action and therapeutic value of veratrine preparations was not very encouraging. All statements agree that the action of the different members of the veratrine series is a complex one, resembling that of aconite and that their effect on the blood-pressure is the resultant of the action on the various factors determining blood-pressure. With small doses they produce a stimulation of the medullary centres. Owing to the stimulation of the cardio-inhibitory centre, the heart is slowed and its output decreased. This, however, is counteracted by vasoconstriction due to the stimulation of the vaso-motor centre, so that a rise of blood-pressure results. With larger doses the vagus nerve endings are paralysed and the pulse becomes quicker, while at the same time the vaso-motor centre is depressed, so that the blood-pressure may be somewhat

¹ Haultain: *Edinburgh Medical Journal*, October 1913, *British Medical Journal*, September 26, 1914, p. 537.

lowered. So far as the therapeutic uses of veratrine are concerned Cushny states that "neither its pharmacological action nor therapeutic experience supplies any indication for its internal use." *Veratrum viride*, since its activity is due to veratrine, according to Cushny,² might well be discarded from the pharmacopeia. "*Veratrum album* is also a superfluous drug." Meyer and Gottlieb appear to hold the same view for they do not refer at all to the action of the members of the veratrine series on the circulation of the intact animal.

The question is further complicated by the fact that there is a considerable difference of opinion concerning the identity or non-identity of the active principles present in the various species of the genus *veratrum*. All are agreed that the active principle of *veratrum Sabadilla* the alkaloid veratrine, is different from that of *veratrum album*, which owes its activity to the presence of protoveratrine. But as regards *veratrum viride* opinions differ widely. According to the statement of Cushny quoted above, *veratrum viride* owes its activity to the presence of veratrine. Kobert³ also does not distinguish between the action of the different species of *veratrum*. According to him they all have the same action: the blood-pressure is first raised owing to a direct peripheral action on the walls of the blood-vessels. This effect is independent of the vaso-motor centre, which with larger doses becomes paralysed, so that the blood-pressure then is lowered.

Cash,⁴ on the other hand, holds that *veratrum viride* contains only traces of veratrine and owes its activity to the presence of another alkaloid, veratroidin. According to this authority *veratrum viride*, through the action of veratroidin, produces a slowing of the pulse and a fall of blood-pressure mainly through stimulation of the cardio-inhibitory centre. With larger doses stimulation of the cardio-inhibitory mechanism yields to depression, leading to an acceleration of the pulse and a rise in blood-pressure.

² Cushny: Textbook of Pharmacology, 5th edition, 1910.

³ Kobert: Lehrbuch der Intoxikationen, 1906.

⁴ Cash: Textbook of Pharmacology, edited by Hale White, 1901, p. 168.

A different standpoint is taken by the United States Pharmacopeia which identifies veratrum viride and veratrum album by designating both by the term "veratrum." If veratrum viride owed its action to the presence of protoveratrine, as veratrum album undoubtedly does, one would expect, from the observations of Watts Eden on the action of protoveratrine, that the administration of veratrum viride would produce the same effects on blood-pressure as protoveratrine. These effects are according to Watts Eden⁵ a preliminary fall of pressure which is suddenly transformed into a rise together with a more rapid heart-beat. The blood-pressure remains at the higher level for 3 to 4 minutes and then gradually falls, to remain constant at a level slightly lower than the original blood-pressure before the injection. If the vagi are cut the preliminary fall of blood-pressure is absent and a marked rise together with a more rapid pulse are the immediate results of the injection. The inhibitory nerve-endings of the vagus are paralysed, while small doses produce a stimulation of the vaso-motor mechanism; larger doses paralyse it and then produce a fall in blood-pressure.

Whether veratrum viride contains protoverafrine, does not seem to be definitely known. But Wood⁶ strongly protests against the identification of veratrum viride and veratrum album by saying that: "The medical practitioner who wishes to produce a profound influence with veratrum viride should always *order and see that he gets*" (the italics are his) "not the veratrum of the U. S. P. but veratrum viride." Wood also emphasises the distinction between the action of veratrum viride and veratrine. According to him "veratrine is not the active principle of veratrum viride and has no physiological relation to it."

The account of the action of veratrum viride given by Wood is essentially the same as that given by Cash. Wood emphasises that the fall of blood-pressure obtained by small doses of the drug is due solely to the slowing of the pulse produced by stimulation of the cardio-inhibitory centre. The statements of Wood and Cash are based mainly on the experimental investigation of

⁵ Watts Eden; Archiv f. Exp. Patholog. u. Pharmakologie, 29, 1892, p. 440.

⁶ Wood, H. C.: Therapeutics, 14th edition, 1908, p. 262.

H. C. Wood, Sr. and of Löwensohn (working under Kobert). These two papers have not been accessible to me, but it appears from the articles of Wood, Jr. and Cash quoted above that Wood, Sr. and Löwensohn studied the action of the alkaloids veratroidine, jervine and pseudojervine which had been obtained from *veratrum viride*.

A recent paper by Houghton and Hamilton⁷ on the action of a proprietary preparation obtained from *veratrum viride* does not add to our knowledge as this paper refers to only three experiments on dogs with confusing and contradictory results for which no explanation is offered, and which certainly give no justification for the therapeutic use of the drug.

This brief survey is sufficient to show the confusion with which the question concerning the pharmacological action and the identity or non-identity of the active principles in the various members of the veratrine series is at present surrounded. The only point on which there appears to be an agreement is that they are therapeutically useless.

EXPERIMENTAL PART

The following observations were made with two different preparations obtained from *veratrum viride*. One was prepared in the laboratory as follows: 15 grams of finely powdered *veratrum viride* were extracted with 100 cc. alcohol 96 per cent with frequent shaking for 6 hours. The extraction was repeated twice for somewhat longer periods (16 hours), 100 cc. alcohol being used for each extraction. The united alcoholic extracts were evaporated on the water bath. A dark brown resinous mass with a characteristic portwine-like aromatic odour remained behind. This residue was rubbed up with water made faintly acid with acetic acid and allowed to stand over night. The filtered extract on neutralisation gave a precipitate which dissolved almost completely on adding a few drops of acetic acid. The solution was again filtered and made up with water to 60 cc., so that 1 cc. of this watery extract corresponded

⁷ Houghton and Hamilton: *Therapeutic Gazette*, 1905.

roughly to 0.25 of veratrum viride. This preparation will be referred to as the "laboratory preparation."

The approximate strength of the extract was estimated by determining the total solids. 1 cc. extract contained 12.2 mgm. total solids. The other preparation was a proprietary preparation which was obtained commercially. This preparation is a watery solution of the active principle of the drug and is stated by the makers to be of such a strength that "1 cc. represents 4 grains of the drug and is equivalent to 20 minims of tincture veratrum viride B. P. 1885." This preparation will be referred to as the "proprietary preparation." On evaporation it was found that 1 cc. of this preparation contained 32 mgm. total solids. If this preparation were therefore diluted 1:100, 3 cc. of this diluted preparation contained 1 mgm.

These figures are of course not a measure of the amounts of the active principle of the drug present in the preparations. In fact of the two extracts the one prepared in the laboratory while having essentially the same effect as the proprietary preparation, produced a more lasting and marked effect if compared with equal quantities of the proprietary preparation. The figures for the total solids are merely an indication of the maximal amount of the active principles administered at each injection. The significance of the dosage will be referred to later.

Most of the experiments were made on fully grown cats under chloroform. The weight of the animals varied with few exceptions from 2.5 kilos to 3.5 kilos and the doses given in this paper refer to the dose actually given to an animal, and are not calculated per kilogram of animal. A few experiments were made on rabbits. Artificial respiration was not used unless this is specially stated. The injection was made into the external jugular vein and the blood-pressure taken from the carotid artery. Doses of 2 mgm. to 6 mgm. of the laboratory preparation and of 5 mgm. to 15 mgm. of the proprietary preparation if injected intravenously, produce most varied and irregular effects. There are almost always profound disturbances of the respiration, sometimes amounting to complete standstill, at other times the stoppage of the respiration is interrupted by convulsive

breathing. The blood-pressure often shows only a slight transient fall and remains on the normal level or may even rise. Sometimes, however, the blood pressure falls at once to almost zero, but the heart continues to beat slowly, until the animal dies of respiratory failure; if artificial respiration is induced the blood-pressure rises again to or above the normal level. When convulsive breathing follows the injection, the blood-pressure may show great fluctuations of 100 mm. of mercury or more accompanying the respiratory movements. The pulse is sometimes slowed; more frequently it is quickened. In all cases subsequent injections have very little or no effect unless very large doses are given. Artificial respiration if begun before the injection is made appears to interfere with the action of the drug on the blood-pressure; frequently there is a slight transient rise in blood-pressure under these conditions. The inhibitory nerve endings of the vagus are in all cases paralyzed. Generally speaking, the effects of such large doses on blood-pressure are quite irregular and cannot be analysed readily. The effects just described correspond fairly well with the accounts of the action of veratrine and protoveratrine given in the literature and referred to above.

Very different and constant effects are obtained, however, when smaller doses of the drug are administered and its mode of action then becomes clear. Doses of 0.2 mgm. to 1.0 mgm. of the proprietary preparation and of 0.1 to 0.25 mgm. of the laboratory preparation produce the following results (see figs. 1, 3, 4, 5). The respiration is slowed and may be completely arrested for a short time. The blood-pressure falls at once and remains at a very much lower level for a prolonged period from 10 to 30 minutes. It then gradually rises and reaches again its normal level. The fall of blood-pressure is as a rule accompanied by slowing of the pulse, but, in cats, at any rate, this effect is sometimes absent or, at any rate, not very marked (fig. 1). Usually there is first a fall of blood-pressure without inhibition of the heart, the slowing of the pulse appearing a short time (10-30 seconds) after the blood-pressure has established itself on a lower level. In one case this slowing of the pulse occurred

as late as 5 minutes after the injection, while the blood pressure fell immediately after the injection. It is evident then that the fall of blood-pressure is not due to the slowing of the heartbeat, although naturally this slowing of the pulse, if it does occur, adds to the effect. This conclusion is further confirmed by the fact that when the inhibitory nerve-endings of the vagus are paralysed by atropine, veratrum viride will still produce a marked fall of blood-pressure without diminishing the rate of the heartbeat. Or if atropine is injected after vera-



FIG. 1. Experiment 5. Cat, 2 kilo. Effect of small dose of veratrum viride (prop. prep.) 0.16 mgm. The tracings from below upward represent signal marker, time tracing (10 seconds) base line, also flow of urine, blood pressure, respiration. Note that there is no marked slowing of the pulse.

trum viride has produced a lowering of blood-pressure and a slowing of the pulse, the latter effect disappears but the blood-pressure remains low. In an experiment in which the volume of the intestine was recorded at the same time, a marked dilatation occurred after the first injection of veratrum viride; a further dilatation took place when the cardio-inhibitory effect was eliminated by a subsequent injection of atropine.

It will be noted that the effect of a small dose of the drug

on the respiration and blood-pressure is similar to that of stimulating the central end of the divided vagus. That indeed is the explanation of the action of *veratrum viride*. For if both vagi are divided before injecting the drug no effect is produced on either respiration or blood-pressure (fig. 6). It may be stated here too that there is also no slowing of the heart under these conditions. If one vagus remains intact the drug will still produce its typical effect. It follows then that the drug in small

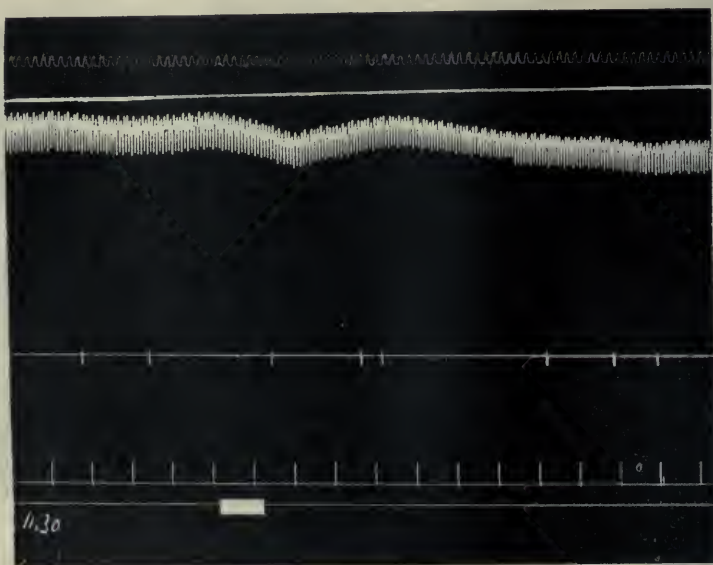


FIG. 2. Same experiment as figure 1. Effect of record small dose given 15 minutes after first dose.

doses exercises its effect on blood-pressure neither peripherally through an action on the vessel walls or the vaso-motor nerve-endings nor through a direct action on the vaso-motor centre or the heart, but reflexly through stimulation of the afferent vagus fibres. The action on the respiration is also produced reflexly. With regard to the slowing of the rate of the heart beat it can be stated with certainty only that it is not due to a stimulation of the cardio-inhibitory nerve-endings or to a direct

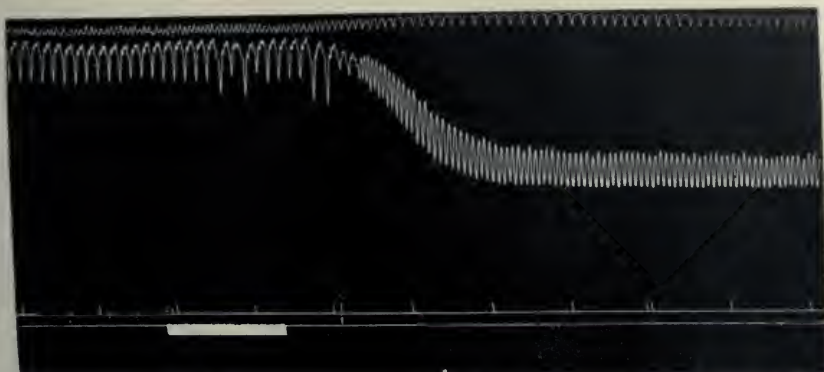


FIG. 3. Experiment 23. Cat. Effect of small dose of veratrum viride (lab. prep.) 0.12 mgm. The tracings from below upward represent signal marker, time marker (10 seconds) and base line, blood pressure, respiration. In this experiment a second dose gave a similar although less marked effect. Note the marked slowing of the pulse.

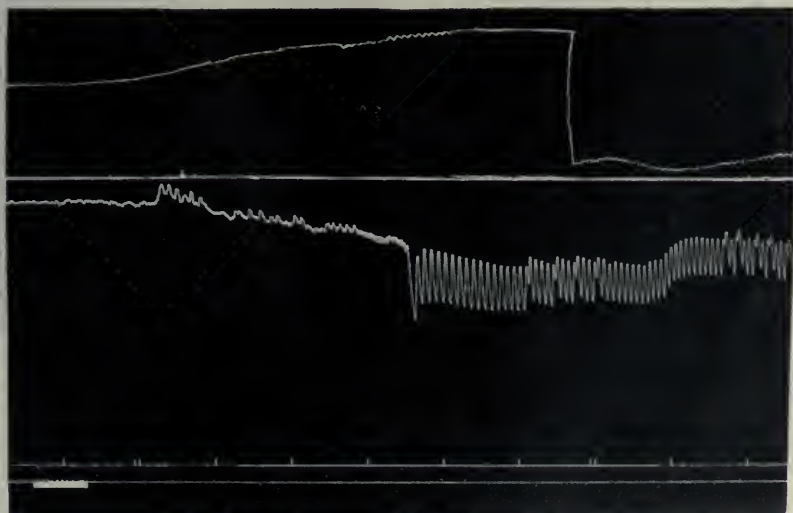


FIG. 4. Experiment 33. Cat, 2.7 kilo. Effect of small dose of veratrum viride (prop. prep.) 0.6 mgm. on blood pressure and intestinal volume. Tracings from below upward represent signal marker, time marker, and base line, blood pressure, volume of intestine. The lever of the piston had to be lowered in the middle of the record owing to the dilatation of the intestine. Respiration stopped 30-40 seconds after the injection, but was resumed spontaneously after about half a minute.

action on the heart muscle. Whether the drug acts directly or reflexly on the cardio-inhibitory centre will be discussed later.

This conclusion is confirmed by the following observations: Plethysmographic records of the volume of intestine show a marked dilatation accompanying the fall of blood-pressure (fig.

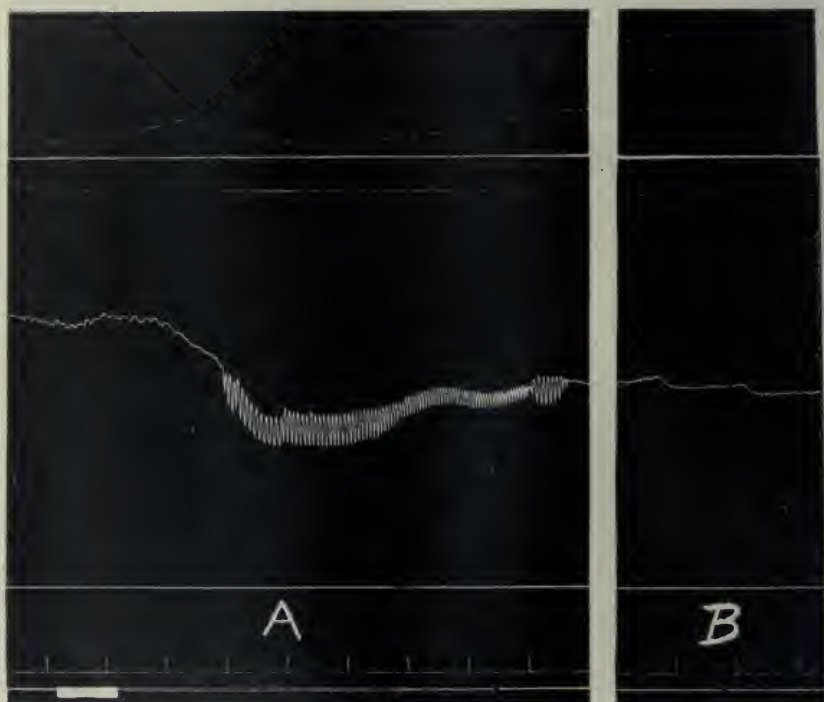


FIG. 5. Experiment 17. Cat. Effect of small dose of veratrum viride (prop. prep.) 0.6 mgm. on blood pressure, respiration and kidney volume. Tracings from below upward represent signal marker, time tracing (10 seconds), base line, blood pressure, respiration, kidney volume. Note initial shrinkage of kidney and subsequent expansion. An interval of one minute between A and B.

4). The kidney volume shows a shrinkage at first, but this is frequently followed by a marked expansion so that eventually the kidney volume is larger than before the injection (fig. 5). The same effect on the kidney volume may be observed on cats when the central end of the divided vagus is stimulated. In

rabbits this effect of dilatation of intestine and shrinkage of kidney was observed by Bayliss as the result of stimulating the depressor nerve.

That the dilatation of the blood-vessels and the fall of blood-pressure produced by veratrum viride is not due to a paralysis of the vessel wall or of the vaso-constrictor nerve-endings is, apart from the reasons given above, evident also from the fact that after veratrum viride has produced its effect adrenalin and pituitrin are still capable of inducing as marked a rise of blood

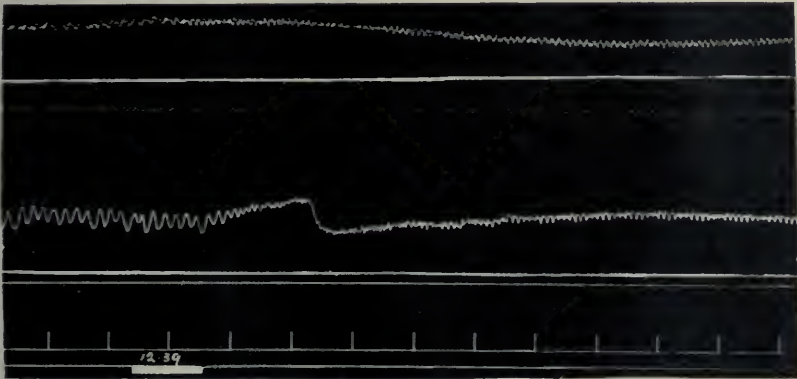


FIG. 6. Experiment 18. Cat. Both vagi cut. Injection of small dose of veratrum viride (prop. prep.) 0.6 mgm. Tracings from below upward represent signal marker, time marker (10 seconds), base line (= 100 mm. mercury), blood pressure, respiration, kidney volume. The record demonstrates that veratrum viride is inactive if both vagi are cut.

pressure as in a normal animal. As no experiments on this point appear to be on record, it was ascertained experimentally that in a rabbit in which the blood-pressure has been lowered by stimulation of the depressor nerve, adrenalin will produce as great a rise of blood-pressure as in a normal animal, even while stimulation of the depressor nerve is being carried out.

The effects just described are observed only when a small dose of veratrum viride is given for the *first* time. If after the blood-pressure has been allowed to return to its normal level and the respiration has become normal again a second small

dose of the drug is given from 20 to 60 minutes after the first dose no effect is produced on either blood-pressure or pulse or respiration. There is sometimes a slight slowing of the respiration or a slight fall of blood-pressure, but if such effects occur they are very transient and insignificant compared with the results of the first dose. A third small dose has also no effect and larger doses may now be injected without producing the severe respiratory disturbances which usually follow such doses when injected into a fresh animal. *Veratrum viride* therefore paralyses the mechanism through which it acts. It paralyses with great readiness the afferent nerve-endings of the vagus after first stimulating them. The other parts of the mechanism are not markedly affected, for stimulation of the central end of the divided vagus still produces the typical effect: stoppage of respiration and fall of blood-pressure, although the latter effect may sometimes be absent.

The efferent cardio-inhibitory nerve-endings of the vagus are not so readily paralysed by *veratrum viride* and it is interesting to remember that they are also not stimulated directly by the drug, since, as stated above, no slowing of the pulse is observed if the vagi are divided before the drug is injected. The first small dose of the drug does not, as a rule, paralyse the cardio-inhibitory nerve-endings. Stimulation of the peripheral vagus may sometimes produce a less complete inhibition after a small dose of the drug than it did before, but even such an effect may be absent. Two or three small doses or one large dose are necessary to paralyse the cardio-inhibitory nerve-endings.

It has already been stated that a large dose injected after the afferent vagus nerve-endings have been paralysed by a small dose, does not produce the profound disturbances which follow when a large dose is injected into a fresh animal. There may be no stoppage of respiration, there may even be no lasting effect on blood-pressure. Frequently a marked rise in blood-pressure, which may persist for sometime, follows the injection of a large dose. The rate of the heart-beat may be increased owing to the paralysis of the cardio-inhibitory nerve-endings. But the rise in blood-pressure is certainly not entirely due to this factor

as it may occur with the vagi divided. The origin of this pressor effect has not been investigated in detail. It is probable, however, that large doses of the drug act directly on the medullary centres. The respiratory centre, at any rate, is affected directly by large doses of the drug, which will sometimes produce a slowing or stoppage of respiration even when the vagi are cut.

It is now possible to understand why large doses of the drug give such irregular and apparently paradoxical results. For in such doses *veratrum viride* may produce any of the following effects. A stoppage of respiration and a dilatation of the blood-vessels through the afferent vagus nerve-endings; or it may at once paralyse them. It may slow the heart through stimulating the cardio-inhibitory centre or may paralyse the cardio-inhibitory nerve-endings and then increase the heart-beat and raise the blood-pressure. And lastly with such doses it will act directly on the medullary centres. It is not surprising that the resultant effect of so many antagonistic actions is irregular.

It has been stated above that the action of the drug has essentially the same effects as stimulation of the central end of the divided vagus. It is generally assumed that in cats the fall of blood-pressure produced by stimulation of the central end is due to stimulation of the depressor nerve which is supposed to be bound up in the vagus trunk in cats. It was therefore to be expected that in rabbits where this nerve is separate, cutting of the vagi would not prevent *veratrum viride* from lowering the blood-pressure, as long as the depressor nerves were intact. Conversely injecting the drug after cutting the depressor nerves and leaving the vagi intact should have the effect that respiration should be slowed or arrested, while vaso-dilatation should not occur. But this is not the case. In rabbits with vagi cut and depressor nerves intact *veratrum viride* (in small doses) produces no effect whatever. If, however, the depressor nerves are cut and the vagi remain intact the drug has the usual effect on blood-pressure and respiration (vaso-dilatation and stoppage of respiration). The assumption then that the drug acts on the respiratory centre through the vagus and on the vaso-motor through the depressor is not correct. We must conclude that

both centres are acted upon reflexly through the vagus. The significance of this conclusion will be discussed later.

The fact that a small dose has no effect when the vagi are cut or when the vagus nerve-endings have been paralysed by a small dose injected previously shows that the drug in small doses has no direct action on the heart or on the blood-vessels. With larger doses such an action becomes apparent, but has not been further investigated.

Incidentally it may be stated here that after atropine a dose of *veratrum viride* which in a normal animal produces the typical effect on respiration and blood-pressure may be without effect and a larger dose must now be given in order to produce slowing of respiration and vaso-dilatation. This indicates that atropine paralyses to a certain extent also the afferent vagus nerve-endings. The same conclusion has been arrived at by Dreser^{*} on other grounds.

Since clinically the administration of the drug in eclampsia is stated to induce diuresis the secretion of urine was recorded in a number of experiments. In some experiments the injection of *veratrum viride* was followed by an increased flow of urine, more frequently, however, there was no such action. It has already been stated that the immediate effect of the drug is a shrinkage of the kidney volume, which is sometimes, but not always, followed by a marked expansion. The experimental evidence then, gives no indication, that *veratrum viride* has any specific diuretic action on the kidney, similar to that, for instance, of pituitrin. And it is not necessary to assume such an action in order to explain the diuresis observed clinically in eclampsia. The suppression of urine in eclampsia is presumably due to the extreme vaso-constriction in which the blood-vessels of the kidney participate. When this condition is relieved as the result of the vaso-dilatation produced by the drug the conditions for the secretion of urine are reëstablished.

^{*} Dreser: quoted from Schmiedeberg *Pharmakologie*, 1902, p. 132.

ON THE INTERRELATIONSHIP OF THE MEDULLARY CENTRES

It has been shown in the preceding pages that veratrum viride produces vaso-dilatation through the afferent fibres of the vagus itself. This conclusion is not in agreement with accepted ideas as stated in text-books. According to these the vaso-dilatation produced by stimulating the central end of the cut vagus in cats or dogs is due to stimulation of the depressor nerve fibres which in these animals are supposed to be bound up in the trunk of the vagus. There are, however, on record some old observations carried out in von Bezold's laboratory by Dreschfeld⁹ which show that in rabbits stimulation of the central end of the vagus produces an even greater fall in blood-pressure than stimulation of the depressor itself. Further support to the conclusion arrived at in this paper is given by some observations of Brodie.¹⁰ On investigating the effects of stimulating the oesophageal, cardiac and pulmonary branches of the vagus, Brodie found that stimulation of the pulmonary branches produced besides stoppage of respiration a marked fall of blood-pressure accompanied by inhibition of the heart. If the inhibitory effect was excluded by a previous administration of atropine, there was only a fall of blood-pressure due to vaso-dilatation. Stimulation of the cardiac and the oesophageal branches of the vagus was comparatively ineffective. The action of veratrum viride is therefore strikingly similar to that produced by electrical stimulation of the pulmonary branches of the vagus.

Another remarkable feature of the action of the drug is the close parallelism of its action on the respiratory centre and on the vaso-motor centre. An inspection of the tracings accompanying this paper show how the fall of blood-pressure develops *pari passu* with the slowing of respiration. The same parallelism is shown in the ineffectiveness of a second dose: there is either no effect on either blood-pressure or respiration or if, as

⁹ Dreschfeld: Untersuchungen aus dem physiol. Lab. Wuerzburg, I, 1867, p. 326.

¹⁰ Brodie: Journal of Physiology, 26, 1900, p. 92.

sometimes happens, the second dose still produces a distinct effect, its action extends to both circulation and respiration. Further, if the afferent nerve-endings are partially paralysed by atropine, a very small dose, which is efficient in a normal animal, has no effect on either blood-pressure or respiration. If the dose is now increased the action on respiration and blood-pressure develops again equally. All these observations suggest that the point of attack of the drug is simple and not double, that there is in the afferent branches of the vagus not one set of nerve-endings connected with the respiratory centre and another connected with the vaso-motor centre but that the stimulation of one nerve-ending affects both the respiratory centre and the vaso-motor centre. If this is so, we must assume that a central connection exists between the respiratory and the vaso-motor centre, so that impulses arriving at the respiratory centre can be communicated to the vasomotor centre.

The fact that the effect of the drug on the respiration may pass off more rapidly than the effect on the circulation does not speak against such a view. For the effect may persist longer in the one centre than in the other owing to the fact that the respiratory centre is more easily stimulated to resume its normal function than the vasomotor centre.

It has further been shown that the drug acts also on the cardio-inhibitory centre and the question arises now whether it affects this centre directly or reflexly, since the slowing of the pulse is certainly not produced by a stimulation of the cardio-inhibitory nerve-endings. Now if *veratrum viride* acted directly on the nerve-centre one would expect that a second small dose should still produce a slowing of the pulse. This, however, is not the case. It is not possible to assume that the cardio-inhibitory centre is paralysed by the first small dose, and that the second small dose is ineffective for that reason, since the rate of the heart-beat may be quite normal when the second dose is injected. We must conclude then, that the cardio-inhibitory centre also has a central connection with the respiratory or the vaso-motor centre and that the drug slows the pulse reflexly through its action on the afferent (pulmonary) nerve-endings

of the vagus. That the vaso-motor and cardio-inhibitory centres can be influenced by impulses passing centrally from the respiratory centre, has been suggested before as an explanation of certain phenomena observed in connection with the influence of respiration on circulation. A conclusive demonstration of such a close relationship between the three medullary centres has, however, been wanting, since the experimental conditions under which it could be demonstrated were too complicated and introduced other disturbing factors, such as lack or excess in oxygen or in carbonic acid or alterations in the mechanical conditions affecting the circulation in the thorax. The selective action of veratrum viride in small doses on the afferent pulmonary nerve-endings of the vagus makes it possible to demonstrate without the introduction of other disturbing factors the inter-relationship between the three medullary centres.

ON THE IDENTITY OR NON-IDENTITY OF THE ACTIVE PRINCIPLE
OF VERATRUM VIRIDE WITH VERATRINE OR
PROTOVERATRINE

The effect of small doses of veratrum viride is quite different from the action of protoveratrine as described by Watts Eden⁵ and of veratrine as given by Lissauer.¹¹ On the other hand the accounts of these observers of the action of veratrine and of protoveratrine respectively on the circulation might be taken as a fairly accurate description of the action of large doses of veratrum viride, when one considers that the effect of such doses is very irregular. Compared with the amounts of the crude extracts of veratrum viride used in these experiments, of which the active principle is of course only a fraction, the doses used by Lissauer and Watts Eden in their investigations are indeed large. The observations by Lissauer were made with doses of 0.4–0.8 mgm. of the pure alkaloid pro kilo, those of Watts Eden on protoveratrine with 0.05–0.1 mgm. of the pure alkaloid. Moreover, both these observers worked with curarised animals and artificial respiration and we have seen that artificial respira-

¹¹ Lissauer: Archiv. f. exp. Pathol. u. Pharmacologie 23, 1889, p. 36.

tion modifies the action of *veratrum viride*. On the other hand, we find, in the older observations of v. Bezold and Hirt¹² an account of the action of the alkaloid *veratrine* which in many points resembles the action of small doses of *veratrum viride* as given in this paper, while it differs in a great many respects from the account given by Lissauer. The observations of Bezold were made with doses from 0.2 mgm. to 2.5 mgm., so that the discrepancy between the results of Bezold and of Lissauer cannot be due to the factor of dosage.

It is evident then that before the question of the identity or non-identity of the active principles of *veratrum viride* with either *veratrine* or *protoveratrine* can be decided a reinvestigation of the two last-named alkaloids is needed. I had intended to carry out such a reinvestigation, but the present circumstances have prevented me from doing so.

ON THE THERAPEUTIC ADMINISTRATION OF THE DRUG

Since there is still some uncertainty concerning the active principle of *veratrum viride* and since in any case the active principle is not, as yet, obtainable commercially, extracts of the drug have to be employed. An official preparation suitable for subcutaneous injection does not, however, exist, so that a definite dose cannot be fixed. From the observations recorded in this paper it is evident that the drug should be given in such doses that it will just produce a marked lowering of blood-pressure and that there is no advantage of increasing the dose beyond this point. The effects of a large dose given intravenously have been described in this paper; it may sometimes lead to a collapse of the animal and death from respiratory failure, frequently the effect of a large dose is to paralyse at once the mechanism through which it acts, so that then there is no marked effect on blood-pressure, the drug protecting in a way the animal against its own action, or there may be a rise of blood pressure owing to a direct action on the vaso-motor centre. In patients

¹² Bezold und Hirt: Untersuchungen aus dem physiol. Lab., Wuerzburg, I, 1867, p. 95.

when the drug is given subcutaneously and the absorption is necessarily slow, the conditions, as regards the effects of an overdose, will probably be somewhat different. As the result of the slower absorption, a large dose will in every case produce a lowering of blood-pressure and slowing of the pulse, the protective effect of the drug against itself may not come into appearance, and the rise of blood-pressure may take place as an after effect. Owing to the absence of a standardized preparation it is probable that in the past the drug has frequently been given in too large doses in which it is useless, if not dangerous. That perhaps accounts for the fact that veratrum viride in spite of occasional recommendations by clinicians has never come into general use. It is important to bear in mind that the drug is therapeutically valuable only in small doses and that "increasing the dose" may defeat the therapeutic object for which the drug is given. It is tentatively suggested here that the effect on the respiration may be a useful guide and that the drug should be given in such doses that no marked permanent slowing of the respiration takes place. I am informed by Dr. Haultain that with the proprietary preparation $\frac{1}{2}$ cc. is sufficient to produce the therapeutic effect.

SUMMARY

Veratrum viride in small doses has a selective action on the afferent (pulmonary) nerve-endings of the vagus. In cats it thus produces reflexly slowing or stoppage of respiration and a fall of blood-pressure due to vaso-dilatation. As a rule there is in addition a marked slowing of the heart-beat produced reflexly through vagus-inhibition, but in cats this effect is sometimes absent. These effects of small doses are dependent on the integrity of the vagus nerves.

The drug after having stimulated the afferent nerve-endings of the vagus, paralyses them so that a second or third dose is without effect.

With larger doses the drug in addition to the effects just mentioned, paralyses the cardio-inhibitory nerve-endings of the

vagus and has also a direct action on the medullary centres leading to vaso-constriction and to paralysis of respiration. These additional effects are not dependent on the integrity of the vagus nerves.

Since large doses of the drug have so many diverse and partly antagonistic actions, the general result of a large dose of veratrum viride is very complex and irregular.

The manner in which veratrum viride acts reflexly on the medullary centres leads to the conclusion that impulses arriving at the respiratory centre can be communicated to the vaso-motor centre and to the cardio-inhibitory centre and that a central connection exists between these medullary centres.

The question whether veratrum viride owes its action to veratrine or to protoveratrine or to another alkaloid is discussed and left undecided. It is pointed out, however, that in the light of the present observations, the pharmacological action of veratrine and protoveratrine requires reinvestigation.

Veratrum viride is therapeutically valuable, as in suitable doses it affords a means of producing a lasting vaso-dilatation through acting reflexly on the vaso-motor centre.

ACTION OF DRUGS ON THE ISOLATED GALL-BLADDER

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The present investigation was undertaken with the hope that an examination of the effects of various drugs on the gall-bladder would suggest some remedial measures which might prove of clinical value in the treatment of biliary colic. The medical treatment of gall-stone colic is usually limited, as far as drugs are concerned, to morphine and atropine. To relieve the pain, large amounts of morphine are frequently required, doses so large that there is a distinct danger of respiratory depression. It is to counteract this depression that atropine is administered. Neither of these drugs is usually credited with a direct action on the gall-bladder itself; indeed, very little is known concerning the effect of drugs on the biliary vesicle, nor, for that matter, is our knowledge of its anatomy and physiology very definite.

HISTORICAL

The first accurate investigation of the nervous control of the excretory apparatus of the liver was reported by Heidenhain (1). He experimented on dogs, and found that stimulation of the spinal cord in the cervical region caused first an increase, then a decrease, in the flow of bile. He concluded that both these effects were due to the contraction of the entire excretory apparatus. The contractions of the gall-bladder expelled its contents and caused the initial increase in flow. The contraction of the intrahepatic ducts increased the pressure against which the bile must be secreted by the liver cells and so led to lessened secretion.

To Doyon (2) belongs the credit of first recording graphically the rhythmic contractions of the gall-bladder. He introduced through the fundus of the biliary vesicle a balloon filled with water and connected with a piston recorder. The spontaneous movements as recorded were slow and irregular in their rhythm (3). Stimulation of the peripheral end of the vagus was generally without effect on the gall-bladder (4). Excitation of the distal end of the splanchnics led to the contraction of the gall-bladder and of the entire biliary apparatus. Afferent stimulation of either vagus caused contraction, while central stimulation of one splanchnic (the other being left intact) caused inhibition of the gall-bladder. He concluded that motor fibers reached the viscus by way of the splanchnics.

Langley (5) reported that the intravenous injection of suprarenal extract caused an increase in the rate of the bile flow: "When the duct from the gall-bladder is not clamped there is usually a preliminary slowing of the flow of the bile. This slowing is probably due to inhibition of the tone of the gall-bladder so that the secretion passes into this instead of into the cannula."

Freese (6) confirmed Doyon in that both were able to demonstrate spontaneous rhythmic contractions of the gall-bladder. According to Freese, the rate of contraction is quite rapid, ten to twenty per minute. From his experiments he concluded that the splanchnic nerves contained both motor and inhibitory fibers for the gall-bladder, but that the motor fibers predominated. He did not determine the effect of stimulation of the vagi.

Courtade and Guyon (7) confined their attention to the results obtained on stimulating the peripheral end of the vagus. They concluded that the motor fibers for the stomach, intestine, and gall-bladder all passed through the same nerve—namely, the pneumogastric.

Bainbridge and Dale (8), using a modification of Doyon's method, found that stimulation of the sympathetic nerve supply resulted in relaxation of the gall-bladder. They showed that the motor effects described by Doyon (4) and by Freese (6) were not motor effects at all, but were due to the mechanical

pressure on the gall-bladder caused by swelling of the liver and the increased tonus of the muscle due to hyperemia. Stimulation of the vagus nerve increased both the tonus and the rhythm of the muscular coat. Atropine abolished the vagal effect. Stimulation of the central end of the vagus had no influence on the gall-bladder.

Bainbridge and Dale also tested the effects of various drugs on the movements of the gall-bladder. The administration of bile salts, amyl nitrite, or atropine caused relaxation of the muscular wall. Pilocarpine had no direct effect on the gall-bladder; when injected intravenously it caused an apparent contraction due to swelling of the liver; painted on the gall-bladder it did not produce any change in the rhythm. Epinephrine and nicotine caused inhibition and relaxation of the gall-bladder, changes comparable to those occurring after stimulation of the splanchnic nerves.

From this brief review of the available literature it is apparent that the previous workers in this field have arrived at discordant conclusions. It seems probable that the lack of uniformity in their results is due to the many extraneous factors which may influence the gall-bladder when this organ is studied in its normal position. Thus, not only the rate but also the depth of breathing may affect the movements of the gall-bladder. Changes in the circulation following section of the vagi or splanchnics undoubtedly produce changes in the vascularity of the liver as well as of the gall-bladder itself. When these nerves are stimulated the effect on the gall-bladder may be entirely masked by the concomitant circulatory changes.

Dale and Bainbridge, recognizing these disturbing elements, attempted to minimize the effect of the breathing by slitting the diaphragm and carrying out artificial respiration. The changes due to the variations in the liver volume were lessened by carefully dissecting the gall-bladder away from its hepatic attachments. They could not, however, prevent circulatory changes modifying the tracing made by the gall-bladder.

It seemed to us that these complicating factors could be eliminated by studying the excised gall-bladder. Of course, it is

impossible under these conditions to examine the effect of direct electrical stimulation of the nerves but a corresponding effect can be produced by pharmacological agents.

As far as we have been able to ascertain, only one attempt has been made to study the surviving gall-bladder. Doyon (3) reported that if the liver and the gall-bladder be excised and placed in a warm, moist chamber, the gall bladder continues to contract for several hours. He did not attempt to examine the modification of the movements produced by drugs, but cited his experiment as proof that the rhythmic contractions of the gall-bladder are inherent and occur independently of the central nervous system.

METHOD

In these experiments the method employed was as follows: The dogs were killed with ether and the gall-bladder was immediately excised and transferred to a dish containing cold Ringer's solution. The gall-bladder was then opened and the bile and mucus carefully washed away. When a record of the movements of the longitudinal fibers was desired a strip of the entire wall was excised, the incisions beginning at the neck of the gall-bladder and extending to the fundus. The width of the segments was usually from 5 to 10 mm., though the portion derived from the neck of the gall-bladder was necessarily narrower. The strip of tissue was then suspended in a glass vessel containing warm Ringer's solution, and the movements of the muscle were recorded by means of a lever on a slowly-moving kymograph.

For a study of the movements of the circular fibers a ring was cut from the gall-bladder, converted into a strip by an incision parallel to the longitudinal fibers and suspended in the solution in such a manner that the movements of the inner coat were communicated to the recording lever.

No experiments were attempted with the middle coat, which is ill-defined and consists of a few irregularly scattered fibers (9).

NORMAL MOVEMENTS

A few minutes after the preparation has been set up spontaneous rhythmic contractions appear. These movements are fairly regular in rate. At the temperature at which these experiments were carried out (37 to 38°C.) the rate of these contractions was from two to five per minute. Their strength varied considerably during the experiment and seemed to depend upon the tonus of the muscle. Thus, early in the experiment, when the tonus of the muscle was low, the strength of the contractions was very much less than later when the tonus was higher.

In addition to these small and comparatively regular spontaneous movements there were frequently large slow waves which were probably manifestations of changes in tonus and upon which the smaller contractions were superimposed.

A third type of movement was occasionally seen. This consisted of rather powerful but regular contractions, the strength of which waxed and waned rhythmically.

DRUGS WHICH ACT ON THE PARASYMPATHETIC SYSTEM²

The members of this group which have been examined are pilocarpine, physostigmine, and atropine.

Although Meyer and Harnack (10) claim that pilocarpine produces its characteristic effect by stimulating the ganglia of the parasympathetic system, the preponderance of evidence favors the view that this drug stimulates the myoneural junctions of this division of the autonomic nervous system. The action of pilocarpine on the circular muscle of the isolated gall-bladder is shown in figure 1. At the first arrow, 50 mgm. of the alkaloid were introduced into the cylinder, making a concentration of 5:10,000. Almost at once there was a large increase in tonus. During the ascent of the lever the individual contractions could still be made out; these were lost, however, during the maximal effect, so that the muscle was in tetanus. At the second arrow,

² Langley's classification has been adopted.

sufficient atropine was added to make a concentration of 1:100,000. The stimulation was immediately overcome and the gall-bladder relaxed.

Figure 2 is introduced to emphasize the permanency of the effect of pilocarpine. At the first signal, pilocarpine was intro-

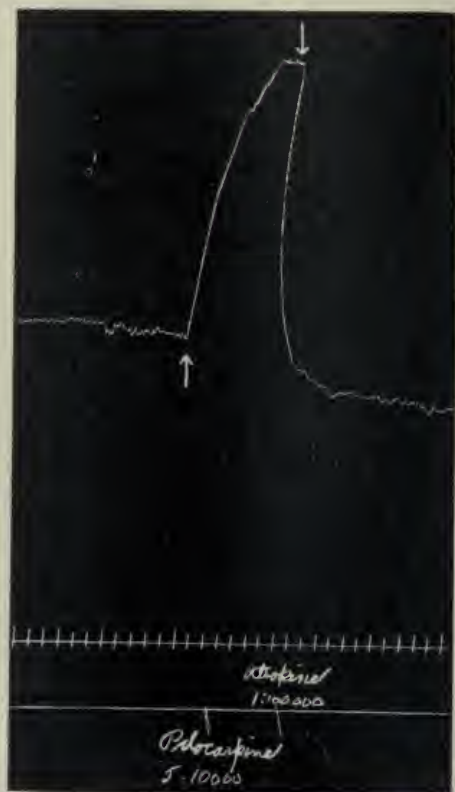


FIG. 1. Gall-bladder. Dog. Circular coat. At the first arrow, pilocarpine to make 5:10,000; at the second arrow, atropine to make 1:100,000.

In this and the subsequent illustrations the upstroke of the recording lever indicates contraction of the gall-bladder; downstroke, relaxation. Time is given in minutes.

duced into the cylinder in such amount as to make a concentration of 5:10,000. It is unusual to find that pilocarpine produces its action in two phases as is here illustrated. Generally, after

the contraction has reached its maximum the tonus remains at the same level for a considerable period, but in this experiment after some five or six minutes the tonus was somewhat lessened though it did not reach normal. It remained at this new level for about an hour, when the effect was removed by atropine. During the increased tonus the individual contractions did not materially change their rate though their prominence was accentuated.

Physostigmine causes an increase in the tonus of the musculature of the gall-bladder; though the rate of the contractions is usually unchanged, in some instances an acceleration has been

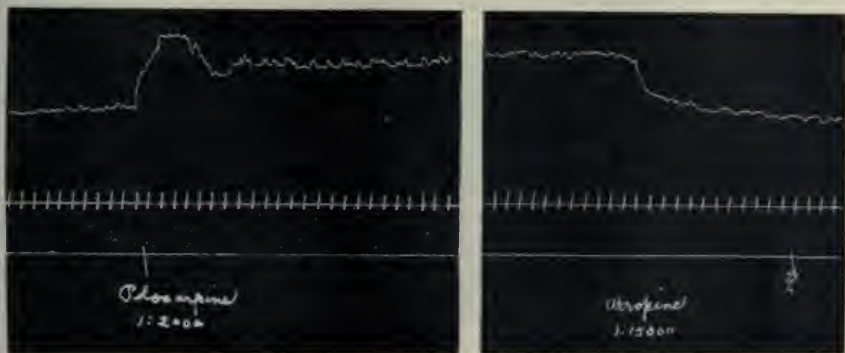


FIG. 2. Gall-bladder. Dog. Longitudinal fibers recording. At A, pilocarpine to make 1:2,000; at B, atropine to make 1:15,000; 40 minutes elapsed between the two charts.

noted. Not infrequently there is a distinct augmentation in the strength of the movements.

The action of atropine on the isolated gall-bladder seems to depend almost entirely upon the degree of tonus which exists at the moment of application. Thus, early in an experiment, when the tonus is low, atropine produces little or no effect. Later, when the tonus has spontaneously increased, atropine leads to a relaxation of the muscle. After a stimulating drug has been applied, the extent of the relaxation will depend not only upon the degree of excitation produced by the drug but

also upon the degree of tonus which existed before its application. Thus, in figure 2, where the tonus was originally low, and where pilocarpine produced only a moderate stimulation, atropine caused a relaxation which was sufficient only to lead to a level of tonus almost identical with that existing during the control period. In figure 1, on the other hand, the original tonus was comparatively high. The effect of pilocarpine was marked, and when atropine was applied the relaxation was so great that the writing point fell below the original level. Here, then, atropine had removed not only the effect of the pilocarpine but had also abolished the preëxisting and spontaneous tonus of the preparation.



FIG. 3. Gall-bladder. Dog. Longitudinal coat. A, epinephrine to make 1:200,000; B, wash; C, epinephrine to make 1:100,000.

DRUGS WHICH ACT ON THE TRUE SYMPATHETIC SYSTEM

Epinephrine

It is now generally recognized that the action of epinephrine is limited to a stimulation of the myoneural junctions of the sympathetic nervous system. It is usually admitted that smooth muscle receives a double and opposed innervation: one set of fibers is derived from the parasympathetic, the other from the true sympathetic system. When one system produces on stimulation increase in function, excitation of the other system produces decrease in function.

This law holds true for the innervation of the gall-bladder. As is shown in figure 3, epinephrine produces changes in rate and in tonus which are the exact opposites of those following pilocarpine or physostigmine. At *A* sufficient epinephrine was added to make a 1:200,000 solution. Almost at once there was a marked loss of tonus with a small decrease in the rate of contraction. At *B* the epinephrine solution was replaced by fresh Ringer's fluid and a gradual recovery to the original rhythm and level of tonus ensued. At *C* epinephrine was again added, this time to make 1:100,000. Again there occurred a loss of tonus. The effect upon the rate and upon the strength of the contractions was more marked than at the first application.

DRUGS WHICH ACT ON GANGLIA

Nicotine

The effect of nicotine on the movements of the circular muscle of the gall-bladder is illustrated in figure 4. The addition of successive doses of nicotine produces no change in the contractions of the gall-bladder until the concentration of 1:5,000 is reached; then there occurs a very slight loss in tonus. There is, however, no marked effect upon the rate nor upon the strength of the contractions. When the concentration is increased to 5:10,000 the effect becomes more marked, and it becomes still more evident when the concentration is raised to 1:1,000. With the higher doses there is a slight reduction in the rate of the contractions and a considerable decrease in their strength.

This depression increased progressively until the nicotine solution was replaced by fresh Ringer's fluid. During the subsequent hour the preparation was washed three times; but, as may be seen from the second segment of the chart, the tonus remained permanently lowered. A striking feature of the late nicotine action is the unusual regularity and strength of the individual contractions. It is, of course, not impossible that the entire action of nicotine is not due to the characteristic effect on the ganglia, but to the general depression which this alkaloid



FIG. 4. Gall-bladder. Dog. Longitudinal coat. A, nicotine to make 1:100,000; B, nicotine to make 3:100,000; C, nicotine to make 5:100,000; D, nicotine to make 10:100,000; E, nicotine to make 20:100,000; F, nicotine to make 50:100,000; G, nicotine to make 100:100,000.

During the hour which elapsed between the two segments of the chart the preparation was washed three times with pure Ringer's solution.

produces on all protoplasm. The picture is certainly quite different from that which nicotine produces on other rhythmic tissue, such as the virgin cat uterus or the intestine. In these there is a sudden and total inhibition of movement with subsequent recovery. In the gall-bladder there is only progressive depression. On the other hand, it seems unlikely that this action is the result of a direct depression of the muscle, for after the removal of the nicotine the contractions are more powerful than under normal conditions, resembling in this respect the exaggerated contractions of other rhythmic tissue when the nicotine stimulation passes into paralysis.

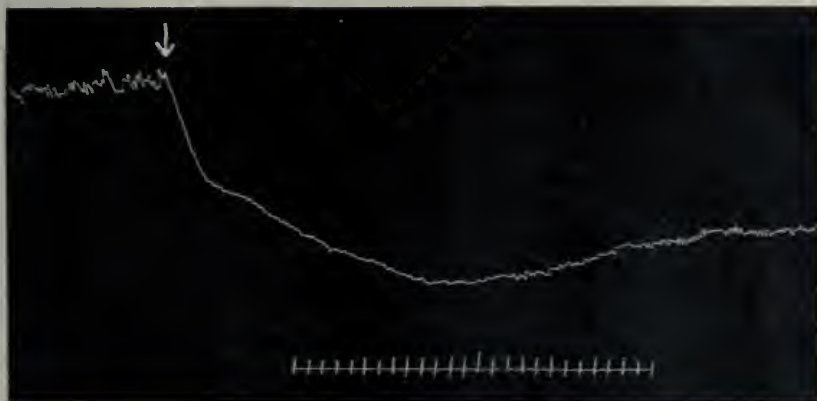


FIG. 5. Gall-bladder. Dog. Longitudinal fibers recording. At the point indicated, spirits of glonoin, 0.1 cc., was added.

DRUGS WHICH ACT PARTICULARLY ON SMOOTH MUSCLE

Nitrites

The effects of nitroglycerine and of the nitrites of sodium and amyl have been examined. Figure 5 illustrates the effect of adding 0.1 cc. of spirits of glonoin to the 100 cc. of Ringer's solution in which the longitudinal strip of the gall-bladder was beating. Immediately upon the addition of the nitroglycerine there occurs a marked loss of tonus which becomes progressively greater during the succeeding half hour. Then a very

gradual return towards the original level sets in. Even during the initial abrupt fall in tonus the spontaneous movements are not completely abolished. During the more gradual decrease and the subsequent increase in tonus, the spontaneous movements though weaker are not materially changed in their rate.

The sodium nitrite produced a similar but less marked effect on the tonus. The rate and strength of the individual contractions was less affected than by spirits of glonoin.

Amyl nitrite produces changes which are almost identical with those of the sodium salt. It may again be pointed out that the effect of the nitrites is almost solely on the tonus and that the strength and rhythm of the contractions are not essentially altered.

Bile salts

Figure 6 serves to illustrate the response of the circular coat of the gall-bladder to bile salts. At the point indicated by the first arrow, 20 mgm. of sodium glycocholate were added to the Ringer's solution, making a concentration of 1:5,000. The typical action is a marked relaxation of the muscle, with a weakening and slowing of the spontaneous contractions. The initial stimulation is unusual and no explanation can at present be offered. At the second arrow, the preparation was washed by passing through the cylinder 200 cc. of fresh warm Ringer's solution. Despite the removal of the bile salts the progressive depression of the muscle was not interrupted. An analogous action occurs on the addition of ox-gall.

One other characteristic feature of all these experiments may be indicated at this point—the rhythmic function of the gall-bladder is extremely persistent and is not readily inhibited by any of the drugs which have been employed. Thus, after nicotine, epinephrine, nitrites, and bile salts, the rhythmic movements of the gall-bladder are not entirely abolished. The chief action of these drugs is upon the tonus and upon the strength of the movements, not upon their rate.



FIG. 6. Gall-bladder. Dog. Circular coat. A, sodium glycocholate, 20:100,000; B, wash; C, strophanthin, 1:2,000.

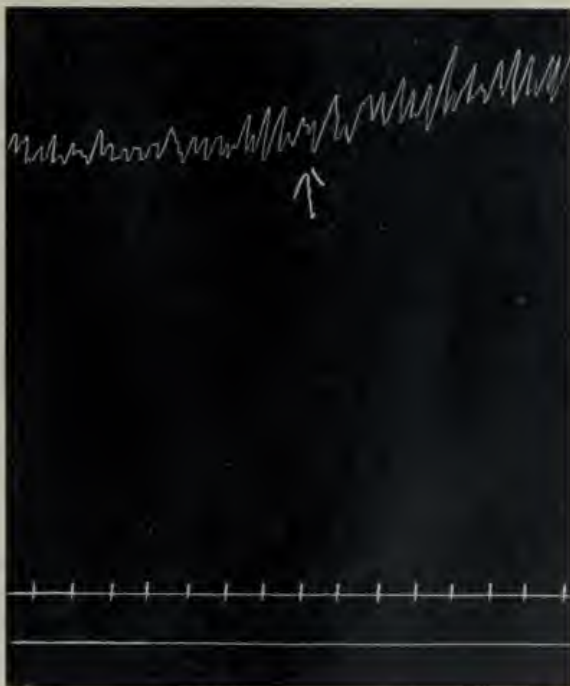


FIG. 7. Gall-bladder. Dog. Circular coat. Morphine 5:100,000.

Strophanthin and barium chloride

Like all other smooth muscle, that of the gall-bladder is thrown into increased activity by the application of either of these drugs. They produce not only an increase in tonus, but also an increase in the rate and in the strength of the individual contractions. The reaction to strophanthin is illustrated in figure 6 (C).

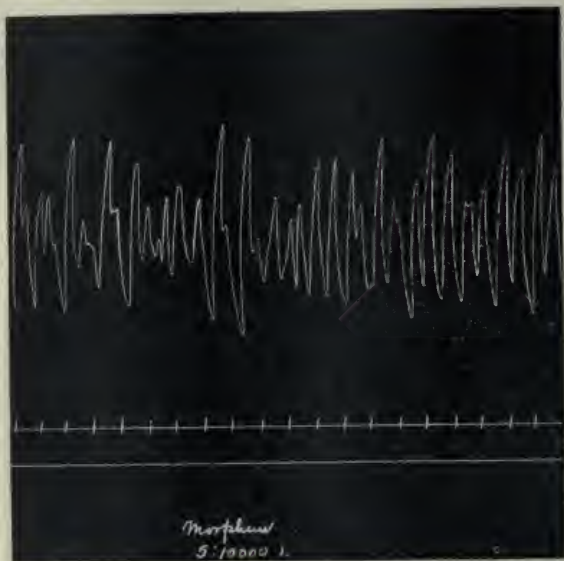


FIG. 8. Gall-bladder. Dog. Circular coat. At the point indicated, morphine to make 5:10,000.

Morphine

The determination of the action of morphine on the gall-bladder was undertaken with considerable interest, for it seemed possible that the prompt relief of the pain of gall-stone colic was due in part to the well-known action of morphine on the pain-receptive area of the brain, and in part to a direct local depression of the gall-bladder itself. The results that have been obtained have proved somewhat contradictory. Thirteen ex-

periments were made in all to determine the action of morphine. In one experiment, morphine in concentrations of 1, 3, and 5 to 100,000, produced a small but undoubted increase in the strength of the contractions. The tonus was heightened but the rate was not modified. This effect is shown in figure 7. This action, however, is atypical, for in all the other experiments morphine in concentrations varying between 1 and 5 to 100,000 produced either no effect at all or a very slight loss in tonus. With these concentrations neither the rate nor the rhythm of the contractions was modified (fig. 8). With concentrations between 5 and 25 to 100,000 there was usually a gradual loss in tonus. With very high concentrations (50:100,000, and above) the rate and the strength of the contractions were usually decreased, the strength being much more affected than the rate. From these experiments it seems safe to infer that morphine in therapeutic doses does not produce a relaxation of the gall-bladder.

CONCLUSIONS

The gall-bladder, like all other smooth muscle, receives a double innervation. The motor impulses are derived from the parasympathetic system, and those drugs which are recognized as stimulants of this system produce contraction of the gall-bladder. Atropine, which paralyzes the nerve endings of the parasympathetic system, produces a relaxation of the gall-bladder in the presence of moderate tonus.

The inhibitory fibers are derived from the true sympathetic system, and epinephrine produces relaxation of the gall-bladder and a slight decrease in the rate and in the strength of its rhythmic movements.

Like all other smooth muscle, that of the gall-bladder is depressed by the nitrites and by bile salts and excited by strophanthin and barium chloride. Morphine in therapeutic doses has in all probability no local action on the gall-bladder.

It is because we appreciate the risk of applying to clinical conditions facts founded on animal experiments that we plan studies of the gall-bladder of man. However, if, as Aschoff

claims, gall-stone colic is due to spasm of the gall-bladder and not of the biliary vessels the following clinical conclusions seem justified:

1. Morphine relieves the pain of biliary colic by acting upon the central nervous system.

2. Atropine and the nitrites would seem to be indicated in the treatment of gall-stone colic.

3. The use of epinephrine to produce relaxation of the gall-bladder is not justifiable, inasmuch as a systemic action can be obtained only after intravenous injection.

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THE EFFECTS OF CHELIDONIN ON SMOOTH MUSCLE

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Chelidonin is the alkaloid of *Chelidonium majus*, which belongs to the Papaveraceae. Its empirical formula, as given by Schmidt,¹ is $C_{20}H_{19}NO_5$, and the melting point 135° . The most soluble salts of Chelidonin are the sulphate and phosphate, the hydrochloride being relatively insoluble.

The systemic effects of chelidonin in intact frogs and mammals were studied by H. H. Meyer² in 1892. In frogs, chelidonin produces a morphin-like successive depression of the cerebrum, cerebellum and medulla; finally, also the spinal cord, but without subsequent irritation. Applied locally, chelidonin paralyzes the skeletal muscle, sensory and motor nerve endings, possessing in this respect a cocain-like action. In mammals a morphin-like narcosis (analgesia and sleep) without marked depression of reflexes is produced. Large doses produce narcosis without nausea and vomiting. A weak indication of the irritation of motor centers and increase of reflexes is seen, slowing of the pulse and finally paralysis of the vasomotor center with large doses and paralysis of sensory nerve endings. The use of chelidonin is said to be accompanied by relatively few side effects.

From this it is seen that chelidonin exhibits certain differences from morphin, namely, (a) lesser toxicity; (b) absence of central stimulation; (c) a diminution or absence of side actions. Accordingly, chelidonin should possess certain therapeutic advan-

¹ Schmidt, E.: Mittl. aus d. pharmaceut. Inst., Univ. Marburg, 1888, 5:15.

² Meyer, H. H.: Arch. exp. Path. Pharm., 1892, 29:397.

tages over morphin. However, before passing to such considerations, further pharmacological study of the effects of chelidonin could be profitably made, for instance, on smooth muscle, as its predominant rôle of action may be more peripheral than central.

The object of the work here presented was to ascertain what effects are produced: (1) by the direct application of chelidonin to surviving organs whose important functional element is smooth muscle, and (2) systemically, upon the functions of these organs (normal and treated) in intact animals. The experiments will be presented in the manner in which they were performed, that is, first those on the surviving organs; then those on intact animals.

1. Experiments on surviving organs

These were performed in the usual manner by direct application of the drug to the excised organ performing spontaneous movements, and recording the same on a slow-moving drum. The organs were freshly excised and suspended in a small vessel of about 15 cc. capacity containing Ringer's solution supplied with a constant stream of bubbling oxygen. The bath was kept at a constant temperature at 37.5° C. for mammalian tissues, and at 28° C. for those of the frog. For circular muscle a small convenient ring from the organ was used, and for longitudinal muscle a short strip. One end of the organ was tied to the arm of a bent glass tube for oxygen and resting on the bottom of the vessel, and the other was suspended to a lightly balanced lever for purposes of recording the movements.

Chelidonin sulphate was used in practically all of the experiments. When this was added to the Ringer's solution, turbidity resulted owing to the alkalinity. However, enough chelidonin remained in solution to exert an effect upon the excised organ. The following organs were studied: oesophagus and fundus and pylorus of the stomach of the frog; intestine of guinea pig, rabbit and cat; vessels of the frog's hind extremities; excised eye of the frog; and lungs of the guinea pig. In each case at least three experiments were performed.

Oesophagus, fundus, and pylorus of frog: The effects of chelidonin on the circular and longitudinal muscle in these organs are illustrated in figures 1 and 2. These consist of depression, since upon withdrawal of the chelidonin by washing spontaneous contractions are again resumed. In the case of the oesophagus these were resumed without the removal of chelidonin.

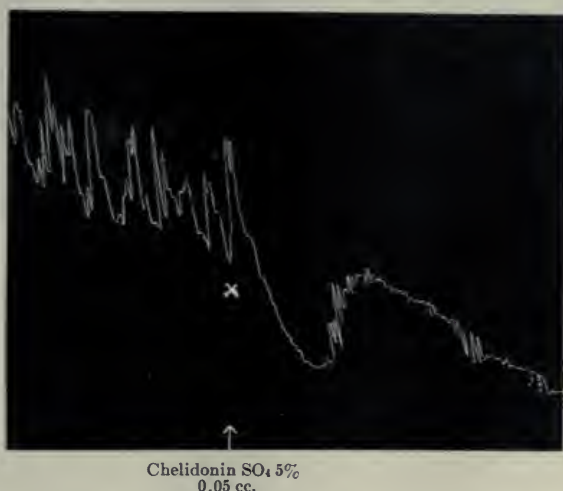


FIG. 1. Ring of frog's oesophagus in Ringer's solution at 28°C. At "x" 0.05 cc. chelidonin sulphate 5 per cent added; not washed out. Peristalsis returns spontaneously.

Mammalian intestine and uterus: The effects of chelidonin on mammalian organs is precisely the same as on those of cold-blooded animals. Figure 3 illustrates the depressant effects of chelidonin on rabbit's intestine; figure 4 on a strip of rabbit's non-gravid uterus. Upon the withdrawal of the drug, peristalsis is promptly resumed.

Effect of chelidonin on various smooth muscle poisons: This consists in a prompt removal of the augmented muscular actions. The results are alike with organs of cold and warm-blooded animals. Further application of the augmentor poison during the chelidonin effect does not bring about a return of peristalsis or tone. An illustration of the effects of chelidonin on the aug-

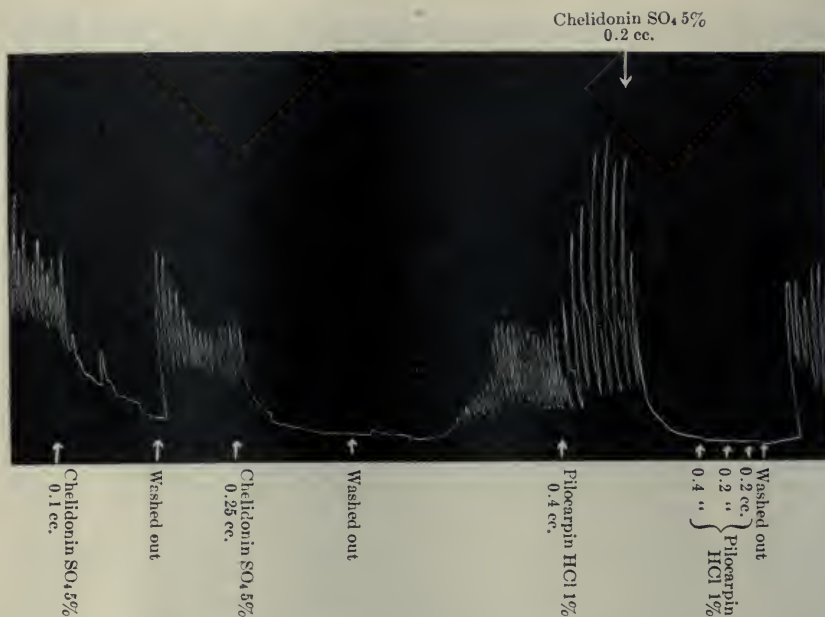


FIG. 2. Frog's stomach—region of fundus; longitudinal muscle. Ringer at 28°C.

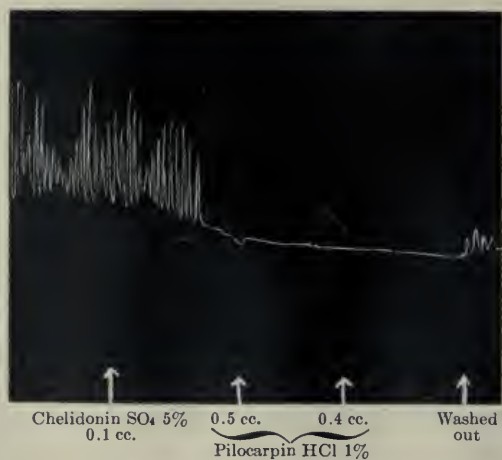


FIG. 3. Ring of rabbit's intestine. Ringer at 40°C.

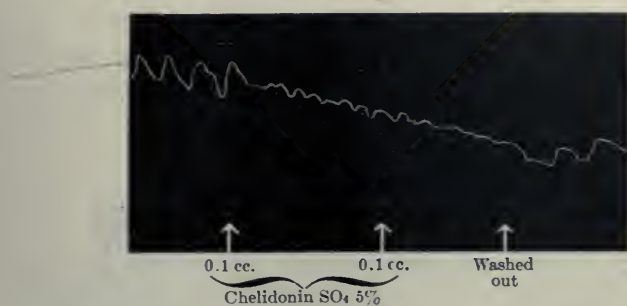


FIG. 4. Strip of rabbit's uterus (not gravid). Ringer at 38°C .



FIG. 5. Ring of cat's intestine. Ringer at 38.5°C .

mentor effects of pilocarpin in the organs of the frog can be seen in figure 2; on those of the cat's intestine in figure 5.

The increased peristaltic effects of pituitrin on a strip of pregnant uterus of guinea pig (fig. 6) were promptly removed by



FIG. 6. Strip of pregnant uterus of guinea pig. Ringer at 38.5°C .

chelidonin. Further application of pituitrin to the chelidonized organ resulted in no change until the poisons were removed by washing, when the organ again responded to pituitrin in the characteristic fashion.

Practically the same may be said of the effects on the marked tonic properties of histamin. This is illustrated in figure 7.

Effect of chelidonin on barium contracture: The prompt removal of the effects of various nerve muscle poisons by chelidonin indicates the possibility of the drug exerting its main action upon the smooth muscle itself. This could be definitely shown by

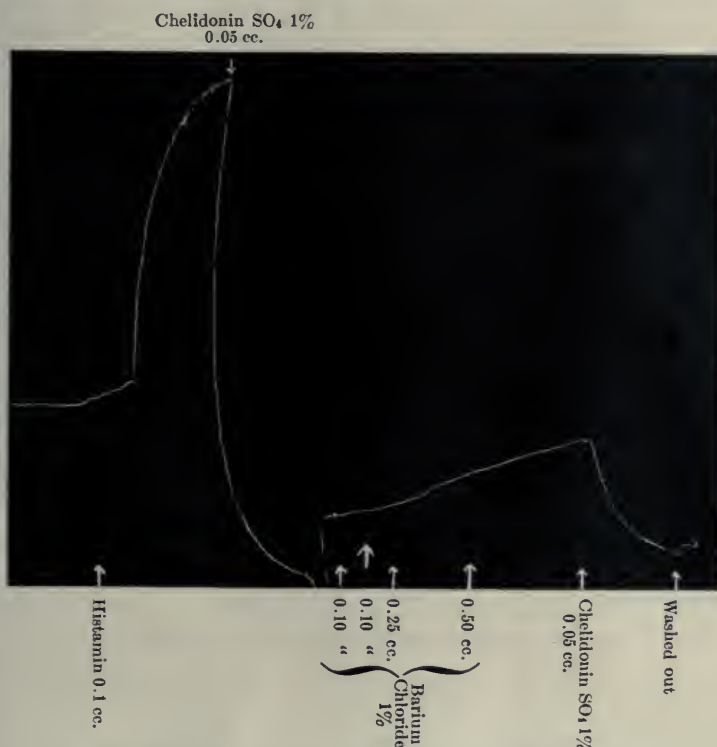


FIG. 7. Strip of pregnant uterus of guinea pig. Ringer at 38.5°C . Previously treated with pituitrin, chelidonin and epinephrin several times; no spontaneous movements.

noting the effect of chelidonin hydrochloride on a barium chloride contracture, since the barium ion exerts its sole action on the smooth muscle. That chelidonin promptly and definitely antagonizes or removes the contracture produced by barium was shown repeatedly with various organs, and is illustrated in figure 8. Removal of the chelidonin by washing resulted

in a prompt return of the increased tonus previously produced by barium.

Bronchial musculature: The effect of chelidonin on this was studied according to the method described by Baehr and Pick³ This consists of perfusing the bronchial circulation of the lungs *in situ* through a cannula tied in the pulmonary aorta and a cannula inserted in the apex of the left ventricle, the lungs being at the same time artificially inflated by means of an apparatus (Meyer's) which permits of spontaneous collapse of the

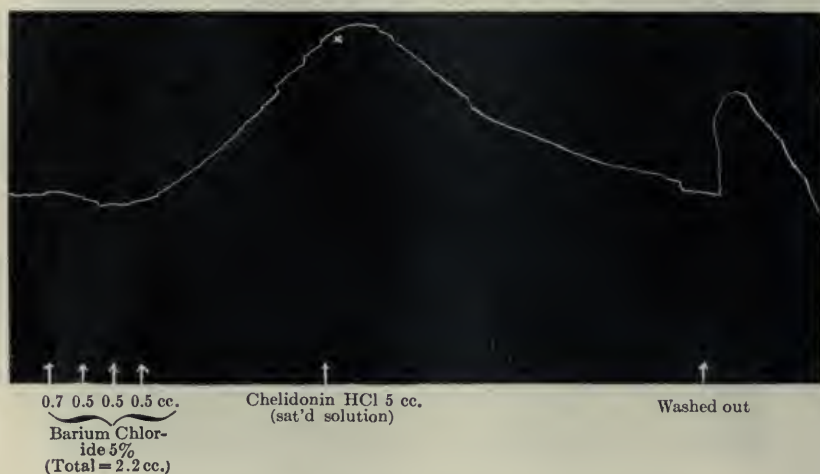


FIG. 8. Ring of frog's oesophagus. Bath at 27°C.

lung during the expiratory phase. When the lungs remain permanently inflated, and no longer collapse spontaneously, it is inferred that the bronchioles are constricted, providing oedema is excluded. A removal of constriction is indicated by a resumption of spontaneous collapse of the lung.

In my work, all perfusion experiments have been made with Ringer's free from sodium bicarbonate on account of the fact that chelidonin is readily precipitated in alkaline solutions.

³ Baehr and Pick: Arch. exp. Path. Pharm., 1913, 74:41.

However, this does not invalidate the results, inasmuch as the observations are comparable. The data of the individual experiments are presented in the following protocols.

EXPERIMENT 1. *Effects of pilocarpin and histamin on surviving lungs previously treated with chelidonin*

TIME	CHANGES IN LUNGS
	Both lungs collapsing well
11:00	Perfusion with chelidonin sulphate 1:1,000.
11:05	Perfusion with pilocarpin HCl 0.05 per cent.
11:10	Lungs collapsing well; no inflation, no bronchoconstriction.
11:20	Perfusion with histamin 1:500.
11:40	Perfusion with plain Ringer's solution for 20 mins., lungs collapsing well.
12:10	Perfusion with histamin 1:15,000.
12:15	Marked inflation and loss of excursions in both lungs; oedema.
12:18	Perfusion with chelidonin sulphate 1:1,000—excursions partially returned; lungs very oedematous.

This experiment indicates that when the lungs are previously treated with chelidonin no bronchoconstriction is produced by pilocarpin and histamin.

EXPERIMENT 2. *Effect of chelidonin on surviving lungs treated with histamin*

TIME	CONDITION OF LUNGS
	Lungs collapsing well; excursions large
4:34	Perfusion with histamin in Ringer (1:30,000).
4:35½	Lungs inflated; no collapsing even when air is stopped or forced.
4:36	Perfusion with chelidonin sulphate 1:1,000.
4:40	Lungs begin to inflate during expiratory phase, first around hilus; excursions small.
4:43	Excursions greater; collapsing more complete; right lung collapses better than left.

Perfusion with chelidonin removes the bronchoconstrictor effects of histamin in the surviving lungs.

EXPERIMENT 3. *Effect of chelidonin on surviving lungs treated with histamin and vice versa*

TIME	CHANGES IN LUNG
9:20	Collapsing well.
9:30	Perfusion of histamin 1:42,000.
9:32	Lungs inflated; no excursions.
9:33	Perfusion of plain Ringer's solution.
9:38	Lung excursions partially return.
9:42	Perfusion of histamin 1:42,000.
9:45	Lungs inflated; no excursions.
9:46	Perfusion with saline.
9:51½	Lungs remain inflated.
9:52	Perfusion with chelidonin 1:1,000.
9:54	Partial collapsing—hilus and edge of lobes.
9:55	More complete collapsing.
10:01	Complete collapsing; excursions large.
10:01½	Perfusion with histamin 1:42,000.
10:05	Left lung collapsing well; right lung getting partially oedematous.
10:06	Left lung collapsing well.
10:08	Left lung collapsing well; right lung slightly due to oedema.
10:08½	Further perfusion with histamin 1:112,000; some fluid expressed from lungs.
10:15	Both lungs collapse well.
10:39	Both lungs collapsing about same as before experiment.

The bronchoconstrictor effects of histamin are removed by chelidonin, but no marked or visible constriction occurs with histamin after chelidonin.

EXPERIMENT 4. *Perfusion of surviving lungs with a mixture of histamin and chelidonin*

TIME	CHANGES IN LUNG
3:35	Excursions good.
3:37	Perfusion with a mixture of histamin 1:112,000 and chelidonin sulphate 1:1,500.
3:46	Entire right lung collapsing well; also lower lobe of left lung.
3:55	Both lungs collapsing well; raised histamin conc. of perfusion mixture to 1:56,000; perfusion begun.
4:12	Both lungs collapsing well; excursions good.
4:35	Both lungs collapsing well; perfusion with Ringer's containing histamin 1:112,000 alone.
4:55	Lungs collapse well.
5:00	Lungs collapse well; experiment stopped.

It is shown that no bronchoconstriction occurs when the lungs are perfused with a mixture of chelidonin and histamin, and the bronchi fail to respond to subsequent perfusion with histamin alone.

It may therefore be concluded that chelidonin relaxes the untreated or normal bronchioles of surviving lungs. Perfusion with saline containing chelidonin produces relaxation of bronchioles previously constricted by histamin, whereas perfusion with saline alone is ineffective. With mixtures of histamin and chelidonin the movements of the lungs remain unaffected. Histamin no longer produces a constriction after the bronchi have been treated with chelidonin.

Pupil: Two eyes of a frog were excised and laid into small conical dishes with the pupil side upward. To one eye a drop of epinephrin (1:1000) was added, and the other was flooded with 5 per cent chelidonin sulphate solution. The pupil of the epinephrin eye dilated widely within a few minutes and the chelidonin eye remained unchanged for 4 hours. When chelidonin was added to the dilated pupil of the epinephrin eye, this remained unchanged during the course of a whole afternoon. From this it appears that chelidonin has no demonstrable effect upon the treated or untreated pupil of the excised eye of the frog.

Blood vessels: Perfusion of the vessels of the frog's posterior extremities was performed by the well-known Låwen⁴-Trendelenburg⁵ method. The untreated vessels shows a definite dilator effect with chelidonin alone (see fig. 9). The results of the experiments on treated vessels are presented in the form of curves in figure 10.

These show that chelidonin promptly and markedly increases the flow through the vessels after previous treatment with epinephrin, which markedly lessened the flow. As compared with Ringer's solution alone the addition of chelidonin increased the flow to the same point (and even higher) in one-half to one-seventh of the time. It is to be concluded that chelidonin removes the constrictive effect of epinephrin more effectively

⁴ Låwen: Arch. exp. Path. Pharm., 1904, 51:415.

⁵ Trendelenburg: Arch. exp. Path. Pharm., 1910, 63:161.

than Ringer's solution alone, and this is interpreted to be in the nature of a more marked dilator effect.

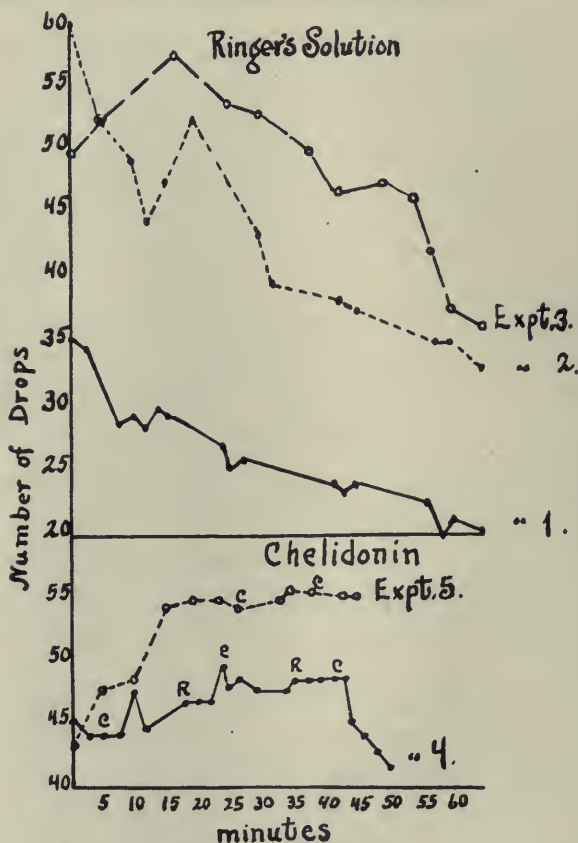


FIG. 9. Perfusion of frog's vessels with Ringer's solution and chelidonin
c refers to chelidonin sulphate 1:1,000.
R refers to Ringer's solution.

2. Intact animals

Intestinal peristalsis: This was observed indirectly through the abdominal wall of the rabbit. For this purpose the animal is fastened to a board on its back and its hair shaved over an area extending between the subcostal margins of the thorax and the iliac fossae. Peristalsis is usually seen immediately as a series of successive serpentine tumefactions of the abdominal

wall in different directions. A wave is frequently seen to begin at a point in the median line or midway between the sternum and the suprapubic region; this passes downward towards the pubis, then it reappears in the left iliac fossa, passes caudad, then directly across the abdomen to the right iliac fossa and upward along the animal's flank until the subcostal margin is reached, then across the median line to the right flank and caudad

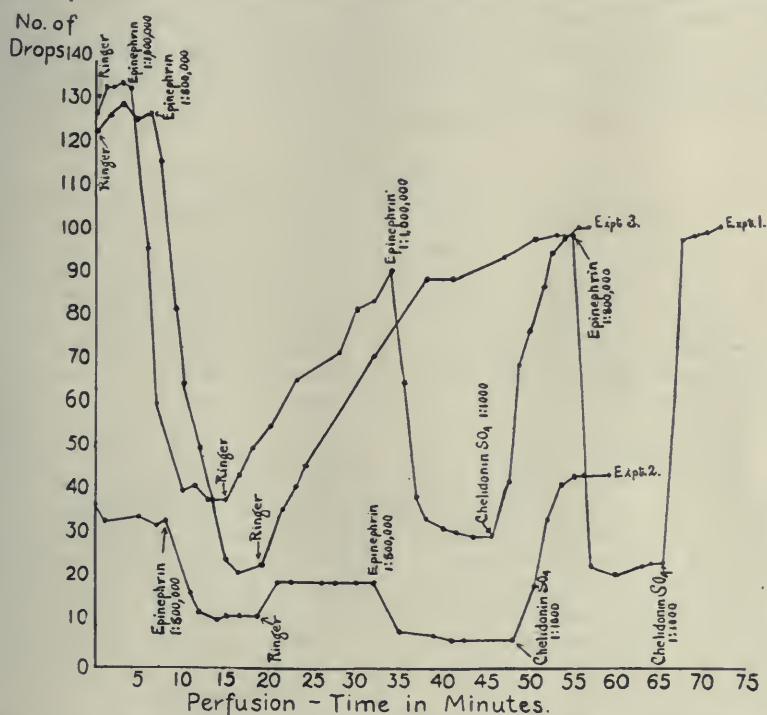


FIG. 10. Effect of chelidonin on frog's vessels constricted by epinephrin

in the direction of the descending colon. These peristaltic waves are usually interpreted as of the colonic type, antiperistalsis frequently being seen in the ascending colon. With small doses of pilocarpin the peristalsis is so markedly augmented that segmentation of the intestine is easily made out. The pilocarpin effect may be likened to an experimental "colic" or "cramps," and is usually accompanied with borborygmus and profuse extrusion of fecal matter.

For each experiment, two rabbits were selected. These were allowed to recover and used for a repetition of the experiment after an interval of a week or so. In one animal, the effect of chelidonin sulphate (2 per cent) on normal peristalsis was observed and the effect of pilocarpin on the depressed peristalsis. Simultaneously in the other animal the effect of chelidonin treatment on the peristalsis augmented by pilocarpin (1 per cent) was observed. The chelidonin was injected intravenously by ear vein (unless otherwise stated), and the pilocarpin subcutaneously. The data have been summarized and converted into the following abridged protocols.

Effects of Chelidonin on Intestinal Peristalsis

CHELIDONINIZED ANIMAL	PILOCARPINIZED ANIMAL
<i>Rabbit 1 (1.4 kgm.)</i>	
<p>Normal peristalsis present; 0.02 gm. chelidonin sulfate abolished peristalsis in 11 minutes; peristalsis slightly returned and further injection of 0.01 gm. inhibited it for about $\frac{1}{2}$ hour.</p> <p>0.002 gm. pilocarpin hydrochloride was injected subcutaneously; no peristalsis in 12 minutes; a further injection of 0.002 gm. pilocarpin brought on peristalsis in 3 minutes.</p> <p>After 35 minutes of marked peristalsis, the movements were diminished by a subcutaneous injection of 0.02 gm. chelidonin.</p> <p>Rabbit recovered.</p>	<p>No spontaneous peristalsis in 1 hour. 0.002 gm. pilocarpin hydrochloride injected subcutaneously: peristalsis began in 10 minutes. In 15 minutes 0.02 gm. chelidonin sulphate was injected intravenously and peristalsis was temporarily depressed for 10 minutes; then gradually augmented; 0.02 gm. chelidonin again injected resulting in temporary depression; finally 0.04 gm. chelidonin was again injected and peristalsis was abolished in 1 hour. Total chelidonin injected intravenously = 0.08 gm.</p> <p>Rabbit recovered.</p>
<i>Rabbit 2 (1.5 kgm.)</i>	
<p>Normal peristalsis present. Intravenous injection of 0.02 gm. chelidonin sulfate depressed peristalsis in 7 minutes, and this lasted for about 13 minutes. Then 0.004 gm. pilocarpin hydrochloride was injected subcutaneously: marked increase in peristalsis in 3 minutes, and required 0.22 gm. chelidonin to abolish this in $1\frac{1}{2}$ hours.</p> <p>Rabbit recovered.</p>	<p>Normal peristalsis present. 0.002 gm. pilocarpin hydrochloride injected subcutaneously: marked increase in peristalsis in 2 minutes, and at the end of 6 minutes 0.08 gm. chelidonin sulfate was injected intravenously: peristalsis entirely quieted in 1 hour.</p> <p>Rabbit recovered.</p>

It may be concluded that chelidonin depresses the normal peristalsis of the rabbit's intestine, and, in comparatively large doses, removes the stimulant effects of pilocarpin.

Effect of chelidonin on movements of the gastrointestinal canal: The following experiments were performed on living cats, in which movements of the alimentary canal were observed with the Röntgen Rays.⁶ The cats were previously hungered and received a thin paste of barium sulphate before the experiment was begun; and during the experiment were suspended horizontally by the back. The results of three experiments are briefly presented in the following protocols.

Experiment 1

CONTROL EXPERIMENT		EFFECT OF CHELIDONIN	
Time	Description of Peristalsis	Time	Description of Peristalsis
	Cat 0.8 kg., June 30, 1914		Same animal as control 2 days later; previously received 0.2 gm. chelidonin and appeared slightly irritated; pupils moderately dilated; tendency to fall to one side.
6:00	Stomach well contracted; deep peristaltic waves and distinct contractions of antrum; whole duodenum filled and shows marked contractions. Prompt passage of gastric contents into duodenum.	5:00	Fed BaSO ₄ meal.
		5:05	Stomach well contracted; deep waves and distinct antrum-contractions; duodenal filling easily visible; with each antrum contraction contents shoot into duodenum and fill a correspondingly greater portion of small intestine; coils of small intestine move continuously.
6:30	Great part of contents in small intestine; stomach well contracted and waves visible; antrum contractions present; also filling of duodenum; the small intestine is filling and shows small movements from time to time.	5:25	Stomach and duodenum empty; whole contents in distal coils of small intestine, and here movements are still visible.

Interpretation: Condition of stomach resembles pyloro-insufficiency clinically; as though there were a pyloro-relaxation in the cat.

⁶ For aid in these experiments, I am indebted to Prof. Dr. A. Fröhlich, and Dr. Eisler of the Radiographic Dept., in the Allgemeine Poliklinik, Vienna.

Experiment 2

5:55	Cat 1.5 kg. Fed BaSO ₄ meal. Immediately after filling, the stomach was contracted; strong peristalsis and segmentation with rapid emptying and filling of small intestine; marked peristalsis of small intestine.
6:00	Chelidonin sulphate 0.3 gm. injected subcutaneously.
6:06	Fundus depressed; antrum contraction still marked; small intestine markedly filled and shows active peristalsis, but without progressive movement of contents.
6:28	Fundus relaxed, and there is rapid emptying of contents through antrum though antrum is not visible clearly; movements of small intestine visible.

Experiment 3

	Cat 1.8 kg. Fed BaSO ₄ meal. Peristalsis in fundus and antrum pylori present.
10:55	Chelidonin sulphate 0.04 gm. subcutaneously.
11:00	Chelidonin sulphate 0.04 gm. subcutaneously.
11:12	Antrum pylori ballooned; larger portions of food passing through pylorus than before chelidonin; appears as though pylorus is more opened; rate and force of peristaltic waves appear to be somewhat increased.
11:25	Chelidonin SO ₄ 0.02 gm. subcutaneously.
11:30	Strong pyloric contractions; marked intestinal peristalsis in small intestine; waves of food shoot rapidly into duodenum; fundus contracts; ballooning of antrum; appears as though sphincter pylori dilates more than before chelidonin was given.
1:30	Fundus and intestine resting; antrum pylori markedly contracted so that a narrow band of constriction is visible in region of antrum.

No definite conclusions could be drawn from Experiments 2 and 3, although there appeared to be a tendency to relaxation of the pyloric end of the stomach in both animals. This effect appeared in about half an hour after injection of chelidonin, and was indicated by the rapid emptying of the pyloric contents. Two hours later, in Experiment 3, the antrum pylori was again contracted, perhaps owing to a wearing off of the chelidonin effect.

Intra vitam effect of chelidonin on bronchoconstriction: In one guinea pig bronchoconstriction (experimental asthma) was produced by the injection of 0.5 mgm. of histamin intraperitoneally. The animal was then treated intravenously with a large dose

of chelidonin, but died within 3 minutes after the chelidonin was administered. At autopsy one lung was markedly inflated, the other collapsed.

Another pig was treated previously with large doses of chelidonin. This was followed by an injection of 0.6 cc. of 1:1000 histamin intravenously. Within 5 minutes the animal was dyspnoeic and died suddenly. At autopsy both lungs were markedly inflated.

No definite conclusions can be drawn from these experiments. Similar and more successful experiments were carried out on rabbits recording at the same time the respiratory excursions.

Effect of chelidonin on bronchoconstriction in rabbits: It is well known from the work of Dale and Laidlaw⁷ and Barger and Dale⁸ that the intravenous injection of about 0.1 mgm. of histamin in an unnarcotized rabbit (about 1.8 kg.) produces almost instantaneous respiratory standstill due to its powerful constriction of bronchial musculature. This is accompanied by a gradual fall of blood pressure and death. The respiration cannot be improved by massage or artificial means owing to the marked bronchoconstriction. This was confirmed in two normal (untreated) rabbits (about 1.5 kg.), each of which received a dose of 0.1 gm. of histamin intravenously. These rabbits served as controls for the experiments on treated animals.

The object of these was to note the effect of the injection of histamin in chelidonized rabbits on the respiration as recorded by an ordinary tambour connected with a tracheal cannula introduced under slight preliminary anaesthesia. The rabbits were previously treated with large doses of chelidonin sulphate (2 per cent) by injection into the ear vein at intervals of 10 to 15 minutes. Short tracings were taken of the respiration before the chelidonin was injected, while the animal was under the effects of chelidonin and with each injection of histamin. Four such experiments were performed with analogous results, which

⁷ Dale and Laidlaw: J. Physiol., 1911, 41:318.

⁸ Barger and Dale: Zent. f. Physiol., 1910, 24:886.

may be illustrated by figure 11 (composed of assembled parts of a long tracing from one experiment.)

As a rule after chelidonin the respiratory rate was somewhat slower though not altered materially in amplitude. There was practically no narcosis, and the reflexes were easily elicited. In all cases practically none, or at the most very slight bronchoconstriction, took place after the first and second doses of 0.1 mgm. of histamin. With higher doses, more constriction was evident as indicated by the respiratory tracings, but in no case did respiratory standstill occur until several times the average fatal dose (0.1 mgm.) was reached. This amounted to 0.3 mgm., 0.4 mgm. and 1.0 mgm. with the respective animals used. The doses of chelidonin administered varied from 0.2 to 0.3 gram intravenously.

Partial success in a similar direction was obtained with the lung plethysmograph in a decerebrate cat. However, owing to the brevity of time and the difficulty of obtaining sufficient chelidonin for experimental purposes at present, this, as well as some other experiments, is left unfinished.

It may be concluded that rabbits previously treated with relatively large doses of chelidonin intravenously do not show bronchial spasm with the same doses of histamin as untreated rabbits.

Discussion

Chemically chelidonin belongs to the isoquinoline group of alkaloids, such as papaverine, narceine and narcotine. Physiologically the effects of these substances on smooth muscle are probably identical, namely, depression. It appears quite certain that the various muscle effects of chelidonin resemble those of papaverine on intestinal muscle as described by J. Pal⁹ and Popper and Frankl.¹⁰

In the Papaveraceae there exists also a physiologically antagonistic group of alkaloids, namely, the phenanthrene group, and

⁹ Pal, J.: Zent. f. Physiol., 1902, 16:68; Wiener med. Wochsch., 1914, 63:1050; Deutsch. med. Wochsch., 1914, 40:164; Deutsch. med. Wochsch., 1913, 39:395.

¹⁰ Popper and Frankl: Deutsch. med. Wochsch., 1912, No. 28.

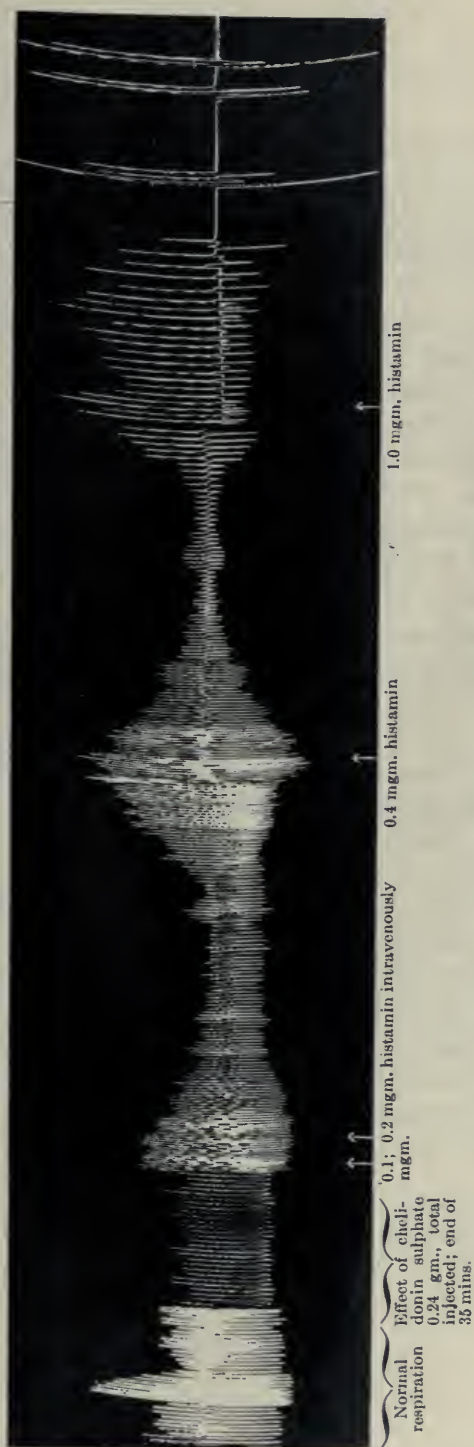


FIG. 11. Effect of histamin on respiration of chelidonized rabbit (2.2 kg.)

these include morphin, codein and thebaine and the derivatives, apomorphin and apocodein. These according to Pal and Popper¹¹ augment the contraction of smooth muscle, particularly in the gastrointestinal tract. The phenanthrene group tends also to produce more striking central effects, such as narcosis and subsequent central irritation, than the isoquinoline group, which possess these actions only to a comparatively mild degree and with much larger doses.

The results of the experiments here presented indicate the efficiency of chelidonin as a depressant of smooth muscle, particularly in the bronchi and gastrointestinal tract. This is brought about promptly and effectively though without paralysis with the doses which were used. This is true of both surviving organs and intact animals. From the practical standpoint this should be of considerable advantage as chelidonin could be used for certain symptomatic relief, such as gastralgia, enteralgia and asthma, and without causing profound central effects.

Ribbing and Rumpf¹² (1893), who have probably published the only extant therapeutic observations with chelidonin, report promising results in this direction. Ribbing and Rumpf made observations on cases of gastric tumor with gastralgia and enteralgia and colic. There was marked relief of pain; no trace of dullness or tendency to sleep, and also very little constipation or othersideactions with doses of chelidonin sulphate ranging from 0.1 to 0.2 gram ($1\frac{1}{2}$ to 3 grains) by mouth. These doses appear small in comparison with those used in animals. Owing to its relatively low toxicity, chelidonin could be given to children with comparative harmlessness, and might to a certain extent replace morphin in pediatric practice.

¹¹ Pal and Popper: *Biochem. Zeit.*, 1914, 57:472.

¹² Ribbing and Rumpf, *cit.*, Meyer, H. H.: *Aerztlich. Verein zu Marburg*, May 16, 1896; *Berl. klin. Wochsch.*, 1896, No. 34.

Summary

(1) Chelidonin promptly abolishes the spontaneous contractions of the following excised organs: oesophagus, fundus and pylorus of the frog's stomach, intestine of cat and rabbit, and pregnant uterus of guinea pig.

(2) Chelidonin removes the effects of pilocarpin, pituitrin, histamin and barium chloride upon surviving organs.

(3) The peripheral blood vessels of the frog previously contracted by epinephrin are more rapidly dilated by chelidonin than by Ringer's solution alone. There is also a definite dilator effect upon the untreated vessels.

(4) The constriction of the bronchial musculature by histamin in the surviving lungs of the guinea pig is removed by chelidonin. Bronchoconstriction does not occur with mixtures of histamin and chelidonin. Rabbits previously treated with large doses of chelidonin do not show bronchial spasm with the same doses of histamin as untreated rabbits.

(5) Chelidonin has no demonstrable effect on the pupil of the excised eye of the frog.

(6) In the living rabbit intravenous injection of chelidonin depresses intestinal peristalsis, and large doses remove the stimulant effects of pilocarpin.

(7) It appears that chelidonin exerts its main effects directly upon smooth muscle.

(8) Therapeutically, chelidonin should prove beneficial in the treatment of such symptoms as asthma, colic and various other enteralgias and gastralgias, and in conditions in which morphin is not well tolerated.

THE COMPARATIVE ACTION OF THE STEREO-ISOMERS OF HYDROXYHYDRINDAMINE

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Hydroxyhydrindamine, $\text{C}_6\text{H}_4 \begin{matrix} \text{CH}_2 \\ \diagup \quad \diagdown \\ \text{CHOH} \end{matrix} \text{CH} \cdot \text{NH}_2$, has been prepared and resolved by Pope and Read¹ into its optically active components. The dextro and laevo isomerides are easily obtained, their salts crystallize well, and are usually sparingly soluble in organic solvents. The bases are solid crystalline substances, they form stable carbonates and liberate HN_3 from its salts. Many of the salts show muta-rotation in alcohol and acetone solutions. In view of the fact that the stereo-isomers of the nicotines,² hyoscyamines³ and adrenalines⁴ differ so markedly in their degree of action, and as the hydroxyhydrindamines are used in the resolution of other substances, it was decided to compare the physiological action of the two isomers. I am indebted to Professor Pope for having given me an ample supply of these substances. The hydrochlorides of the two bases were used; they occur in a white crystalline form readily soluble in water, sparingly soluble in alcohol, and are powerfully optically active.

ACTION ON ISOLATED TISSUES

(a) *Striped muscle.* For this purpose the two sartorius muscles were placed in a Lucas trough and excited by platinum electrodes every five seconds. The two muscles were treated

¹ Trans. Chem. Soc. CI, 1912, 758.

² Berichte d. deutsch chem. Gesellsch., 1904, 37, 1225.

³ Cushny 1904, Journ. Physiol., 30, 176.

⁴ Cushny 1908, Journ. Physiol., 37, 130.

in an identical way, both were immersed in Ringer's solution, the one with 0.1 per cent d.H. and the other with 0.1 per cent l.H. In both cases fatigue ensued earlier than normal, and the dextro-variety was slightly more toxic than the laevo-variety; the muscles ceased to respond in about thirty minutes.

Similar results were obtained with the gastrocnemius muscle, both when the muscle was excited directly and through the sciatic nerve. During nerve excitations however the toxicity of the dextro-compound was more marked.

(b) *On plain muscle.* The hydroxyhydrindamines exert a similar type of action. The automatic movements become gradually less and the tonus is reduced. This was shown for the isolated intestine and uterus of the cat and rabbit. As with striped muscle the dextro-variety was somewhat the more toxic. The action of neither is however very significant since to produce a decided action the drug must be present to 0.02 per cent.

(c) *On cardiac muscle.* Both drugs cause a gradual weakening of the contractions. The method employed was to perfuse the heart of frogs in situ through the portal vein with a 0.1 per cent solution in Ringer. Very gradual and slight weakening of the beat occurred, the dextro-variety being a little stronger than the laevo.

On mammalian isolated hearts the action of these salts can be shown more easily: the systolic weakening with 0.1 per cent is much more decided and a fall in diastolic tonus is evident. The rate of the beat is not appreciably altered though if a very strong solution of the drug is employed slight slowing is obtained. Recovery after the drug has ceased to be perfused is rapid and no permanent injury is inflicted. Again the dextro-variety was slightly the more toxic. In many ways the action on the mammalian heart resembles that of small doses of potash salts.

Both varieties dilate blood vessels: this effect was shown both by perfusion experiments and by the use of the oncometer. The dilatation is as marked in the case of the pulmonary and coronary vessels as in the systemic, and the action is clearly on the muscle.

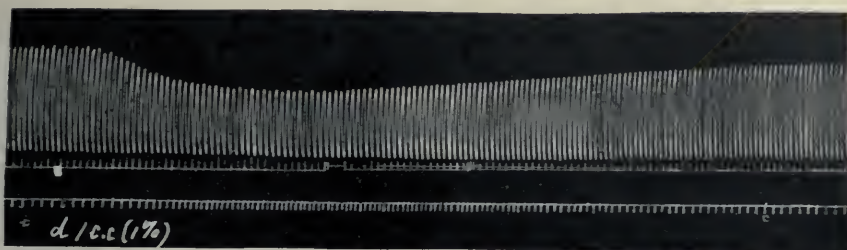


FIG. 1. ISOLATED RABBIT'S HEART PERFUSED WITH LOCKE'S SOLUTION

The second curve represents time in seconds, and the third the drops from the coronary vessels. At the mark 1 cc. 1 per cent dextro hydroxy-hydrindamine was injected into the side tube.

ON MICRO-ORGANISMS

Both isomers exert a mild antiseptic effect though the dextro is more efficient in about the ratio of 3 to 2 than the laevo-variety. This action is considerably less than the effect of lysol or carbolic acid using equivalent amounts, and is of no practical significance. The hydroxyhydrindamine exert no action on haemoglobin or leucocytosis.

ACTION ON INTACT ANIMAL

When these bodies are injected into a vein of an anaesthetised animal, dog, cat or rabbit, very little effect is noticeable. Thus 10 cc. of a 10 per cent solution given to a medium sized dog was without effect on the circulatory system though the cat and rabbit were more susceptible. Thus 2 cc. of a 1 per cent solution injected slowly into the vein of a cat causes a small and temporary fall of blood pressure associated with cardiac weakness and dilatation of vessels. Recovery is rapid and no permanent effect results. The d.H. was slightly more effective than l.H. Respiration became slightly shallower and slower, and this suggested that possibly the drug might exert some action like phenacetine, but injections of 2 cc. into the ear vein of small rabbits were without influence either on the body temperature or respiration or general activity of the animal. I should mention that these injections were kindly made for me

by Dr. Dixon. These experiments make it clear that the hydroxyhydrindamines are relatively innocuous. They exert no specific action on any one tissue like nicotine, adrenalin or hyoscyamine but have a mild depressant action on all forms of living tissue alike.

Camphor⁵ is also a general protoplasmic poison which forms optical isomers and it is interesting to note that, as in the case of the hydroxyhydrindamines, the two isomeric camphors differ less in their action than those of the alkaloids. The l-camphor is rather more toxic than the dextrorotary variety, while the reverse holds in the hydroxyhydrindamines.

CONCLUSIONS

1. The hydroxyhydrindamines exert no specific action but are mild general protoplasmic poisons.
2. Unlike most other drugs having a specific action and which can be resolved into their optical isomers, the isomers of hydroxyhydrindamine show little difference in their action.
3. The d.H is slightly more toxic than l.H. This also is in contrast with drugs exerting a specific action in which the laevo-variety has the more pronounced action.

⁵ Grove 1910, *This Journ.*, 1, 445.

THE ANTITOXIC ACTION OF RATTLESNAKE SERUM ON RATTLESNAKE VENOM WITH A NOTE ON THE PERCENTAGE OF TOTAL SOLIDS OF THE SERUM AND BILE

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In a previous paper¹ we have shown that rattlesnake venom is not toxic for the snake itself. The following series of experiments was planned to show whether the blood serum acted as a detoxifying agent for the venom. The experiments were conducted on pigeons. Except where otherwise stated, mixed solutions of the venom and serum in 0.6 per cent sodium chloride solution were injected into the breast muscles. The results of our first series are shown in Table I. These results are, in practically all cases, very different from those produced when the venom alone is injected. From our venom controls, Tables V and VI, we observed that paralysis usually occurs a few minutes after the injection of the venom. In the experiments in which small quantities of serum were used with 7 mgm. of venom, we had only one fatality occurring within an hour. With larger quantities of serum and 7 mgm. of venom, paralysis usually occurred later and death was delayed for a number of hours. With still larger doses of serum and 7 mgm. of venom, one pigeon, Experiment 10, Table 1, lived 17 days after injection. When we attempted to repeat these experiments with undried serum, Table III, the results did not correspond with those of our former experiments. A closer scrutiny of our work brought out the fact that in the experiments shown in Table I, we had used *Crotalus adamanteus* serum and *Crotalus*

¹ This Journ., vol. vi, No. 5, p. 563, 1915.

atrox venom and that in those included in Table III, *Crotalus atrox* serum and *Crotalus atrox* venom had been used. The effect of mixed injections of *Crotalus adamanteus* serum and *Crotalus adamanteus* venom is shown in Table IV. The results in the experiments with venom and serum from the same species are practically the same as when the venom alone is injected. In Table V, *Crotalus atrox* serum was used with *Crotalus adamanteus* venom, and we observed the same picture of delayed action.

In order to establish the equivalents of the weights of dried serum which we used, we made determinations of the total solids in the serum and also incidentally of the total solids in the bile. The results of our determinations are 13.12 per cent total solids in the blood serum and 14.60 per cent, total solids in the bile.

The number of our experiments is so few that we hesitate to draw any very definite conclusions. We believe, however, that there is a strong indication that each of the blood sera of these two species of rattlesnakes has anti-toxic action for the venom of other species.

EXPERIMENTS WITH SERUM AND VENOM FROM DIFFERENT SPECIES

TABLE I

EXP. NO.	CROTALUS ADAMANTEUS SERUM	EQUIVALENT TO CC. OF SERUM	CROTALUS ATROX VENOM	WEIGHT PIGEON	REMARKS
	<i>mgm.</i>		<i>mgm.</i>	<i>grams</i>	
1	7	0.050	7	402	Partially paralyzed 13 min. after injection. Died 1 hr. 18 min. after injection.
2	14	0.107	7	377	Showed signs of weakness 17 min. after injection. Died 1 hr. 10 min. after injection.
3	21	0.160	7	402	Standing up 4 hrs. after injection. Died following morning.
4	28	0.210	7	377	Signs of paralysis 20 min. after injection. Dead 50 min. after injection.

TABLE I—Continued

EXP. NO.	CROTALUS ADAMANTEUS SERUM	EQUIVALENT TO CC. OF SERUM	CROTALUS ATROX VENOM	WEIGHT PIGEON	REMARKS
	<i>mgm.</i>		<i>mgm.</i>	<i>grams</i>	
5	35	0.169	7	421	Standing up 4 hrs. after injection. Died following morning.
6	75	0.576	7	376	Died the following day about 18 hrs. after injection.
7	100	0.770	7	425	Died following day, about 20 hrs. after injection.
8	100	0.770	7		Died during the night.
9	100	0.770	7		Died during the night.
10	100	0.770	7		Lived 17 days after injection.
11	100	0.770	7		Was killed for autopsy. Injected serum solution 15 min. before venom injection. Died during the night.

TABLE II

EXP. NO.	CROTALUS ATROX SERUM	CROTALUS ADAMANTEUS VENOM	WEIGHT PIGEON	REMARKS
	<i>cc.</i>	<i>mgm.</i>	<i>grams</i>	
1	1.0	10	259	No effect but drowsiness in 2 hrs. 50 min. Died the following morning.
2	2.5	10	308	Paralyzed in 2 hrs. 12 min. Died about 4½ hrs. after injection.
3	5.0	10	377	Paralyzed in 6 min. Standing up 2 hrs. 41 min. after injection. Died the following morning.

EXPERIMENTS WITH SERUM AND VENOM FROM THE SAME SPECIES

TABLE III

EXP. NO.	CROTALUS ATROX SERUM	CROTALUS ATROX VENOM	WEIGHT PIGEON	REMARKS
	<i>cc.</i>	<i>mgm.</i>	<i>grams</i>	
1	0.25	10	290	Paralyzed 6 min. after injection. Died 34 min. after injection.
2	0.50	10	308	Paralyzed 55 min. after injection. Died 1 hr. 10 min. after injection.
3	1.00	10	320	Paralyzed 6 min. after injection. Died 13 min. after injection.

TABLE IV

EXP. NO.	CROTALUS ADAMAN-TEUS SERUM	EQUIVALENT TO CC. OF SERUM	CROTALUS ADAMAN-TEUS VENOM	WEIGHT PIGEON	REMARKS
	<i>mgm.</i>		<i>mgm.</i>	<i>grams</i>	
1	50	0.38	7	277	Died 36 min. after injection.

VENOM CONTROLS

TABLE V

EXP. NO.	CROTALUS ADAMAN-TEUS VENOM	WEIGHT PIGEON	REMARKS
	<i>mgm.</i>	<i>grams</i>	
1	7	386	Died 46 min. after injection.
2	10	371	Paralyzed 5 min. after injection. Died 10 min. after injection
3	10	360	Paralyzed 4 min. after injection. Died 18 min. after injection.
4	10	354	Paralyzed 5 min. after injection. Died 12 min. after injection.

TABLE VI

EXP. NO.	CROTALUS ATROX VENOM	WEIGHT PIGEON	REMARKS
	<i>mgm.</i>	<i>grams</i>	
1	7	393	Paralyzed 2 min. after injection. Died 47 min. after injection.

OBSERVATIONS ON PLASMAPHAERESIS

SECOND PAPER

B. B. TURNER, E. K. MARSHALL, JR., AND PAUL D. LAMSON

From the Pharmacological Laboratory of the Johns Hopkins University

In a previous paper¹ Abel, Rowntree and Turner described a method of removal of plasma from a living animal by repeated bleedings, with return of the corpuscles after they had been washed and separated in a centrifugal machine. This procedure, for which the name plasmaphaeresis was suggested, was found to allow of the removal of very large quantities of plasma without apparent harm to the animal, and the possibility was discussed of its application as a therapeutic measure in various cases, especially such as those in which venesection was already known to have certain beneficial effects and in which the production of anaemia was to be avoided. At the same time the necessity of further study and of guarding against possible dangers was clearly indicated.

The present investigation was undertaken with the twofold object of supplying certain experimental data not contained in the previous paper, i.e., chemical analyses, temperatures, determinations of blood pressure and a continuous series of corpuscle counts, and of acquiring further experience of the permissible limits of bleeding by this method as to rate and total amount removed.

1. CHEMICAL CHANGES IN THE BLOOD AFTER PLASMAPHAERESIS

The character of the changes in the chemical composition of the blood produced by plasmaphaeresis is clearly shown in the following tables, in which the general qualitative agreement of the results is obvious.

¹ Journ. Pharm. and Exptl. Ther. (1914), v, p. 625.

TABLE 1
Plasmaphaeresis for one day, on three dogs

DATE	DECEMBER 21		JANUARY 11		JANUARY 18	
Weight of dog.....	15.1 kg.		11.9 kg.		7.1 kg.	
Blood volume estimated at 7.5%.....	1132 cc.		892 cc.		532 cc.	
Total volume bled and per cent of total blood*	1185 cc. = 105%		1150 cc. = 129%		410 cc. = 77%	
Number of bleedings.....	3		3		5	
Results of analyses	<i>Before After</i>		<i>Before After</i>		<i>Before After</i>	
Percentages	<i>Plasmaphaeresis</i>		<i>Plasmaphaeresis</i>		<i>Plasmaphaeresis</i>	
Total protein of blood.....	22.27	25.23	25.15	25.32	17.34	17.19
Protein of plasma.....	6.62	3.63	6.59	3.17	6.28	3.68
Difference of above ²	15.65	21.60	18.56	22.15	11.06	13.51
Blood counts, millions.....	9.0	11.7	11.8	12.9	6.6	7.5
Total non protein N.....	0.037	0.039	0.029	0.036	0.045	0.055
Urea nitrogen.....	0.016	0.019	0.011	0.017	0.016	0.023
Non urea nitrogen.....	0.021	0.020	0.018	0.021	0.029	0.032
Amino-nitrogen.....	0.0041				0.0048	0.0048

* Blood taken for analysis not included unless made up by equal amount of washed corpuscles from other dogs.

In all cases the protein content of the plasma circulating in the vascular system is lowered by the operation, as might be expected. The extent of the reduction may be taken as a measure of the effectiveness of the procedure in "washing out" the vascular system with saline solution. As is natural, however, the qualitative results are not such as would be obtained in washing out an inanimate system containing the same volume of blood, even when it is taken into account that the plasma removed in the later washings is already diluted by the saline solution used in the earlier reinjections. The result of one day's work in the above experiments, causes, with striking constancy, a reduction of plasma protein to about 50 to 55 per cent of its original value. Three bleedings of one-third volume, with

² This difference, which is inserted to facilitate a rough comparison with the corpuscle count, does not correspond accurately to the proportion of non-plasma protein. To obtain this, the figures for plasma protein, which are percentages of the weight of plasma, should first be recalculated as percentages of the total blood, i.e., they should be reduced about 40 per cent to 50 per cent. The exact figures not being obtainable, as they depend on the relative amounts of blood and plasma, this has not been done. The small change produced in the relative proportions of the corrected figures, as compared with those given, is not important in the present discussion.

return of equal quantity of saline solution should reduce the value to $\frac{8}{27} = 0.296$, that is about 30 per cent, provided there were no renewal of plasma protein. This, of course, is what actually occurs, a fairly rapid flow of protein into the blood taking place from the large store existing in the other tissues. For the same reason, the reduction is slower proportionately as the process is extended over subsequent days. Table 2 shows that the value after three days is about 35 per cent³ of the original

TABLE 2

Continued plasmapheresis on a dog for five successive days January 22 to 26 inclusive.

Weight of dog 8.5 Kg. Estimated blood volume (7.5 per cent) = (640 cc.)
Total blood removed in five days 3335 cc = 521 per cent.

Analytical results in percentage of total blood

A, Before plasmapheresis; B, After plasmapheresis

DATE	A, JAN. 22	B, JAN. 22	B, JAN. 24	B, JAN. 26	FEB. 19
Total protein of blood.....	19.28	19.31	16.81	15.83	11.78
Plasma protein.....	6.38	3.44	2.23	2.92	5.75
Difference of above.....	12.90	15.87	14.58	12.91	6.03
Blood count, millions.....	8.50	8.50	7.50	6.50	3.50
Total non-protein nitrogen...	0.035	0.040	0.037	0.042	0.030
Urea nitrogen.....	0.013	0.019	0.014	0.021	0.012
Amino nitrogen.....	0.0047	0.0056	0.0033	0.0059	0.0038

while the value actually rises slightly in the next two days, showing that the process of restoration has overtaken that of depletion.

In contrast to the above the other factors determined show a constant *rise*. This is not surprising in the case of the corpuscle protein, which varies roughly proportionately with the blood count, the rise of which is discussed elsewhere. That the non-protein nitrogen, and those of its fractional constituents which have been determined, i.e., urea and amino-acids, should

³ A purely mathematical repetition of the first day's reduction to $\frac{3}{10}$ three times, would give $\frac{3^3}{10^3} = \frac{27}{1000}$ or 2.7 per cent. Of course the night interval allows of much greater recuperation than during the course of the operation itself.

also rise as a consequence of plasmapheresis was not anticipated. This rise becomes all the more striking quantitatively when allowance is made for the amount removed, which must undoubtedly exceed the 45 per cent shown to have been removed of plasma protein, even if it does not reach the value of about 65 per cent indicated by theory. The increase is greatest proportionately in the case of urea, where it amounts to about 40 to 50 per cent during one day's work. When this increase is deducted from that shown by the total non protein nitrogen it will be seen that the remainder is nearly constant. Two possible causes suggest themselves for this increase in the urea of the blood, either (1) a reduction of excretion of urea by the kidneys, or (2) an increase in nitrogenous metabolism. As complete studies, on nitrogenous metabolism in connection with plasmapheresis have not yet been carried out, it is impossible at present to decide between these hypotheses.

The results obtained in continued plasmapheresis are shown in table 2. The amount of blood taken was about one volume on each day. The first two columns of analytical results, obtained with samples taken at the beginning and end of the day's work, compare closely with those in table 1. The third column shows the results at the end of the *third* day's work, when the plasma protein reaches the lowest value, 2.23 per cent. The fourth column gives the results at the end of 5 days of plasmapheresis while the last column shows the results 24 days later. Here the plasma protein has gone up again nearly to its original value. The corpuscle protein, and consequently the total protein, also, are low owing to the anaemia. This, as explained elsewhere, was probably due, in part at least, to an extensive infection of one of the wounds. The ineffectiveness of plasmapheresis in lowering the urea and non protein nitrogen of the blood is further emphasized in the results of this experiment.

2. INFLUENCE OF PLASMAPHAERESIS ON THE BLOOD COUNT

In the first experiments on plasmapheresis by Abel, Rown-tree and Turner blood counts were not made till a day or two after the operation was concluded. These were somewhat below normal, indicating, in agreement with the results of Yurevitch and Rosenberg⁴ on rabbits, a slight degree of anaemia, but as the initial figures were not observed the evidence was incomplete.

The present experiments show conclusively that far from lowering the blood count, the first effect of plasmapheresis is uniformly to raise it, often very considerably, while the drop in numbers sets in only after two or three days. The rise in number of erythrocytes may even occur during the withdrawal of blood, and the effect is increased, though not to the degree that would be indicated by purely mathematical calculation, by the return of the corpuscles abstracted. The result of a day's work is with only one exception a distinct rise in numbers: there is usually a falling off again at night, yet the second day may start higher than the first. This cumulative effect however soon gives place to a slight downward tendency, in spite of the periodic increments caused by each plasmapheresis and in an experiment extending over several days the influence of the slowly succeeding anaemia may be evident before the operations are discontinued.

The cause of the paradoxical increase in number of erythrocytes when blood is drawn will be discussed fully in a paper on experimental polycythaemia to appear shortly by one of the present authors (P. D. L.). It may be merely indicated here that it seems to be a general phenomenon induced reflexly by temporary partial asphyxia. Teleologically, its reason is easy to conjecture. By increasing the oxygen carrying capacity of a given volume of blood, the organism appears to attempt to compensate for the diminished volume available for perfusion of the vital organs.

⁴ Russkii Vratch, 1914, p. 637.

An important practical deduction from this observed fact must be emphasized here. As the process of plasmapheresis consists in replacing the original blood by a suspension of corpuscles in a plasma which is more and more dilute in respect of protein, it might be expected that the viscosity of the blood would diminish. As, however, the number of corpuscles has a greater effect on the viscosity than the protein content of the plasma, instead of falling off the viscosity of the blood probably rises considerably, especially in respect of its passage through capillaries of dimensions near that of the corpuscles themselves.

The change in number of white corpuscles, as might be expected, shows a very different picture from that of the red. The effect of plasmapheresis, with few exceptions, is to reduce the number considerably. This is possibly due to the fact that being harder to separate from the plasma in the centrifuge many are lost. Overnight the white count usually rises considerably. It is interesting to note that at the same time the temperature, which has as a rule risen about one degree during the operation, falls to normal. The leucocytosis would therefore appear not to be of infectious origin. A slight febrile reaction has often been noticed from saline injections alone, and it is premature to determine whether the above phenomena can fairly be attributed to the process of plasmapheresis itself. No special effort has been made to investigate this question at present.

In table 4 are shown the variations in the blood count observed in the course of the day, due to individual bleedings. In the few cases where counts were made at the beginning⁵ and end of a bleeding there is a decrease of one-half to two millions. In the interval, while the blood is being washed and centrifuged the count appears usually to rise again, and after reinjection, as already stated, the count will usually be higher than at the commencement. A general tendency to rise throughout the day will be noticed on comparing the figures in the third column: a few exceptions are seen at the ends of experiments Nos. 3 and 5,

⁵ About 10 to 15 cc. was drawn to wash out the cannula before taking a drop for the count.

TABLE 3
Influence of plasmaphaeresis on blood counts and temperature

EXPERIMENT NO.	DATE	VOL. BLEED <i>per cent</i>	REDS, MILLIONS			HAEMOGLOBIN		WHITES, THOUSANDS			TEMPERATURE		REMARKS
			Before	After	Rise	Before	After	Change	Before	After			
1	Dec. 12	60	8.2	8.6	0.4			14	13	-1	37.0	40.5	Overbled
	Dec. 15	141	8.4	11.2	2.8	98	120	63	70	+7	39.5		Died
2	Dec. 21	110	9.0	11.7	2.7	125	142				38.3		Overbled
	Dec. 22	75	9.2	10.5	1.3	108					40.6		Died
3	Jan. 11	128	11.4	12.9	1.5		140						Ether; preliminary operation 2 hrs. before count.
4	Jan. 15	84	9.4	10.5	1.1								Ether; kymograph; collapse; died
5	Jan. 18	76	6.7	7.5	0.8								Ether; kymograph; collapse; died
6	Jan. 22	113	8.5	9.7	1.2						38.5	39.0	See table 4 for intermediate counts and table 5 for after period
	Jan. 23	78	7.2	6.7	-0.5						38.0	38.7	
	Jan. 24	130	6.0	7.6	1.6						38.5	38.0	
	Jan. 25	115	5.2	7.0	1.8						38.5	38.5	
	Jan. 26	85	5.3	8.0	2.7						38.0	40.0	Died of overbleeding
7	Mar. 9	103	8.5	11.8	3.3	115	140	16	13	-3	39.0	40.0	
	Mar. 10	105	10.0	11.0	1.0	130	150	50	33	-17	38.7		
	Mar. 11	34	8.5			128		31			38.5		
	Mar. 12	158	10.3			123		25			38.3	39.5	
	Mar. 15	70	10.0	10.6	0.3	120	145	20	11	-9	38.5	40.0	Collapse; saved Gelatin transfusion died in night
8	Mar. 16	80	10.2	11.3	1.1	127	130	77	27	-50	38.0		
	Mar. 17	81	9.5			120		54			38.7	39.5	
9	Mar. 18	125	9.2			110	120	12					
14	Mar. 22	88	8.0	10.7	2.7	115	125	12	6	-6	38.0	39.0	Collapse; internal haemorrhage; died
	Mar. 23	21	10.1			130		23			38.7		
											38.5	40.0	
15	Mar. 23	59	6.8			97					39.0	40.0	
	Mar. 24	58	7.1			82							
	Mar. 25	59	6.7										
	Mar. 26	53	5.8	6.5	0.7	80	65	35	47	12	38.5		

TABLE 4
Influence of plasmapheresis on blood count. Short interval changes during course of operation. Red counts in millions

EXPERIMENT NO.	DATE	WITHDRAWAL OF BLOOD				RETURN OF CORPUSCLES				REMARKS
		Time	Count before	Volume <i>per cent</i>	Count after	Time	Count before	Volume <i>per cent</i>	Count after	
2	Dec. 21	11.25	9.0	4						4 per cent bled for analysis. Artery prepared. 11.50-12.25 (cocaine). Wound sewed up 5.00. Last count at 5.15. 2 per cent drawn for analysis. Artery prepared 10.00.
		12.33	9.1	44		1.25	9.8	62		
		2.45	10.1	34		4.50		?	11.7	
		4.05	11.2	26				?		
3	Jan. 11	9.27	9.2	0.1		11.45		49	10.5	Ether 11.15. Preparation of arteries and thoracic duct 11.30-1.20. 34 per cent was drawn but half of it returned at once with about two vols. saline (34 per cent). Ether at 2.15. Preparation over 2.40. Ether at 12.05. Preparation of vessels 12.25-35. Saline = 28 per cent injected at 3.01. Saline = 10 per cent injected at 4.23. Saline = 14 per cent injected at 5.30. Dog collapsed at 6.15.
		10.57		42	8.0					
		1.30	11.8	39	9.7	2.22	11.4	56		
		3.05	13.8	39		3.47		56		
4	Jan. 15	4.34	12.9	17		5.35		30		Ether at 2.15. Preparation over 2.40. Ether at 12.05. Preparation of vessels 12.25-35. Saline = 28 per cent injected at 3.01. Saline = 10 per cent injected at 4.23. Saline = 14 per cent injected at 5.30. Dog collapsed at 6.15.
		5.55	12.4	17		6.45		+34		
								22		
5	Jan. 18	3.17	9.4	46	8.9	4.43	7.9	51		Ether at 2.15. Preparation over 2.40. Ether at 12.05. Preparation of vessels 12.25-35. Saline = 28 per cent injected at 3.01. Saline = 10 per cent injected at 4.23. Saline = 14 per cent injected at 5.30. Dog collapsed at 6.15.
		5.29	10.5	38						
		12.48	6.7	27		1.28	5.5	27		
		1.45	7.0	19		2.25		23		
		2.55	8.4	5		3.28		12		Ether at 2.15. Preparation over 2.40. Ether at 12.05. Preparation of vessels 12.25-35. Saline = 28 per cent injected at 3.01. Saline = 10 per cent injected at 4.23. Saline = 14 per cent injected at 5.30. Dog collapsed at 6.15.
		4.10	7.4	14		4.56		18		
		5.11	7.5	12		5.48		13		
		6.07	7.5	3						

6	Jan. 22	11.40	8.5	39				12.50 3.20 5.13	?		
		2.24	9.2	38					39		
		4.14	9.7	31					33		
	Jan. 23	6.50	8.5	5							
		11.28	7.2	31				1.17	36		
		2.21	6.3	20				3.35	24		
		4.00	6.7	27				5.39	?		
	Jan. 24	11.52	6.0	31				1.00	41		
		2.39		33				3.38	36		
		3.54	5.0	31				5.05	33		
		5.44	7.4	29				6.40	33		
	Jan. 25	6.46	7.6	6							
		11.51	5.2	32				2.59	33		
		3.29	5.4	31				4.25	33		
		5.05	6.3	31				6.05	31		
		6.16	7.0	21				6.50	31		
	Jan. 26	12.35	5.3	28				1.28	?		
		2.07	6.0	30				2.54	30		
		3.21	6.5	27				3.49	30		
		5.28	8.0	7							

500 cc. of blood lost by accident. Cor-
puscles replaced from other dog at
5 per cent for analysis.

Lost 30 cc. by accident.
Returned corpuscles from 60 cc. blood
from other dog. Lost 15 cc.

6 per cent for analysis.
Corpuscles from 35 cc. (= 5½ per cent)
other dog's blood added.

3.30 dog collapsed: saved by quick
rejection.
7 per cent for analysis, replaced from
another dog.

TABLE 5

EXPT. NO.	WT. KG.	DATES PLASMAPHAE- SIS VOL. TAKEN	RED COUNT MILLIONS		AFTER PERIOD. MONTHS IN ROMAN FIGURES. DAYS IN ARABIC. RED COUNT IN ARABIC FIGURES. WEIGHTS KG. IN SQUARE BRACKETS. WHITE COUNTS (THOUSANDS) ROUND BRACKETS.										
			BEF.	AFT.	I 27	I 28	I 29	I 30	II 4	II 5	II 8	II 15	II 19	II 23	III 1
6	8.5	I 22-26 521%	8.5	8.0	5.2	4.1	4.7	4.0	2.5	3.6	3.6	4.0	3.6	3.4	4.4
							[7.0]			[6.4]		[5.9]	Hgb. 45%	(46)	[5.8] (46)
15	9.5	III 23-26 229%	6.8	6.5	4.8	4.3	4.3								
					Hgb. 65%	57%	63%								
17	8.2	IV 9 76%	6.5	6.5	(37)	(26)	[9.3]	[8.6]							
					[9.1]										
19	6.3	IV 20 104%	8.3	8.3	6-8	7-9	IV 30								
					Hgb. 85%	Hgb. 107%									
					(10)	(9.3)									
					8.8	7.1	IV 29								
					Hgb. 120%	Hgb. 95%									

and on the second day (January 23) of experiment 6. The last day is the only case observed where the final figure of the day is lower than that of the commencement.

The comparative effect on the blood count of bleeding alone, without return of corpuscles, is illustrated by the following experiment, in which the rise in the count during an interval is clearly seen.

March 20 Dog weighing 8.3 kg. Calculated blood volume 622 cc.

10.06. Hgb. 120 per cent. Reds 10.7 millions.

10.26. Bled 250 cc. = 40 per cent. B.P. fell to 78 mm.

10.23. Hgb. 130 per cent. Reds 8.9 millions. B.P. rose to 140 mm. at 11.27.

11.30. Hgb. 117 per cent. Reds 9.1 millions.

11.45. Bled 110 cc. = 18 per cent. B.P. fell to 94 mm.

11.52. Bled 98 cc. = 16 per cent. Collapse, B.P. 26.

11.57. Hgb. 105 per cent. Reds 8.3 millions. B.P. no longer rises.

1 p.m. Immediately before death Hgb. 100 per cent. Reds 7.3 millions.

It is interesting to note how little the red count has fallen at the time of collapse when 74 per cent of the estimated blood volume has been drawn, and the comparatively high figure at the moment of death.

In table 5 those experiments are collected together where observations have been obtained of the blood counts, weights, etc. in the after period following plasmaphaeresis. The small number of experiments available is due to many accidents, as explained elsewhere. The longest record, experiment No. 6, is unfortunately complicated by an infection of a wound in the dog's leg which gradually became very serious. It is quite possible that the anaemia observed is largely due to this cause. The dog's resistance was evidently lowered. He lost weight and ultimately showed symptoms suggesting distemper and on March 1 was chloroformed, 24 days after the end of the operation. In contrast to this, experiments 17, and 19 show little change in the number of erythrocytes, after 9 to 21 days. Experiment

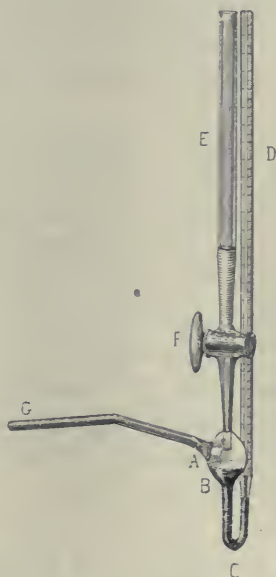
15 showing a moderate degree of anaemia after 3 to 5 days is one of the cases referred to on page 135.

It is obvious that further experiments are needed before the question can be considered as settled whether the falling off in the red cell count some days after plasmapheresis, which was observed in the original paper, is really due to the procedure itself or to accidental complications.

3. CHANGES OF BLOOD PRESSURE IN PLASMAPHAERESIS

Experiments have been performed, using a kymograph attached to the femoral artery, and operating under ether anaesthesia, to determine the effect of plasmapheresis on blood pressure. These did not prove satisfactory. Apart from the effect of the anaesthetic, the decided drop in pressure when blood was drawn caused the strongly alkaline anticoagulant used in the connecting tube to leak into the circulation producing great depression. This could be partly prevented by clamping the artery before bleeding and lowering the pressure in the manometer before opening again, below that anticipated. A continual slight intermixture of anticoagulant with circulating blood through lateral branches due to the pulsations, was still suspected. To avoid this a simple, non-recording manometer was devised by one of us (B. B. T.) Though not new in principle this proved so handy and useful in practice that it is briefly described. A small bulb *A* holding about 5 cc. is connected at the bottom with a narrow tube *B C D* about 30 cm. long and $1\frac{1}{2}$ mm. internal diameter, which is bent upwards in a U-shape just below the bulb, at *B*. To the top of the bulb is attached a small funnel *E* which may be conveniently furnished with a stop-cock *F*. It is desirable, to facilitate washing to make the lower end of the funnel project a few millimeters into the bulb. Connection with the artery is made by the side tube *G*, bent slightly, first up and then down, as shown, about 3 mm. diameter and 15 cm. long. The U-tube is filled with mercury and by means of the funnel physiological saline solution is introduced into

the bulb and allowed to flow out of *G*, expelling all air bubbles before it.⁶ The stopcock being closed the end of the tube *G* is pushed into the arterial cannula, previously furnished with a short rubber connecting tube and the artery unclamped. As the surface of the mercury in the bulb *A* is about 100 times as great as that in the stand pipe *CD* (since the diameter of *A* is 15 mm. or more) the former remains practically stationary after the arterial pressure has once been admitted, all the oscillations being observed in the latter. These may therefore readily



be estimated by eye to within about 5 mm., even when, as is usually the case, the pulse pressure observed is many times greater than that recorded by an ordinary kymograph. As the great weight of mercury in the latter undoubtedly damps down the pulse wave by its inertia, it is probable that the large oscillations observed with the present instrument come much nearer representing truthfully the actual changes in the artery.

One correction only is needed, i.e., for the capillary depression of the mercury in the narrow tube. This, which may be ob-

⁶ This is easily managed by slightly tilting the point of *G* upwards.

served once for all by the initial position of the surface of the mercury in *C* compared with that in the bulb at *B*, amounts with the dimensions given, to about 5 mm.

This little apparatus, which is readily sterilizable, is so easily and quickly connected and disconnected, and the time required for a reading is so short, that there need be no fear of the blood clotting in the mean time. Where it is desired to keep it attached to an artery for any length of time, it may be filled with hirudin solution, at small expense of material. When attached it is kept full, the liquid being retained in *G* by capillary force, and may be kept sterile for use if held in a clamp by *E*. A millimeter scale for reading the pressure may be enclosed in a wider tube fused to the tube *CD*.

With this apparatus many readings of blood pressure have been taken before and after bleeding, and also before and after return of corpuscles. The figures given, unless otherwise stated are *mean systolic pressures*, obtained by averaging the estimated maximum pressure reached at systole over at least one respiratory period. They probably exceed somewhat those which have been obtained with a kymograph in those cases where the pulse pressure was large, since the damping of the latter already referred to, would bring both systolic and diastolic pressures nearer to the mean blood pressure. The relative course of the changes in blood pressure produced by plasmapheresis, will not be greatly affected.

These changes are well illustrated in experiment No. 6 the protocol of which is summarized in the following:

- January 22. Dog 8.5 kg. Estimated blood volume 637 cc.
11.12. Prepared left femoral artery under cocaine anaesthesia.
11.35. Blood pressure with new apparatus, 208 mm.
11.40. Bled 250 cc. 11.45, B.P., 165 mm.
12.50-1.00. Reinjecting corpuscles. 1.04 B.P., 202 mm. Dog allowed to run around. Very lively.
2.00. Dog on table, quieter. 2.12 B.P., 115 mm.
2.24-37. Bled 250 cc. from jugular. 2.48 B.P., 65 mm.
3.13. B.P., 95 mm. 3.20-33. Reinjecting 250 cc. 3.36 B.P., 135 mm. Dog off table. Somewhat depressed. Vomits. On table 4.12.

- 4.13. B.P., 140. 4.14-4.26, Bled 200 cc. (?) 4.31, B.P., 65.
4.54. B.P., 85. 5.13-5.24, Reinjecting 170 cc. 6.30, Reinjecting 50 cc.
6.50. Drew 35 cc. for analysis. 6.52 B.P., 115 mm.
January 23. Bled from veins, 495 cc. in three portions. Omitted blood pressures to economize arterial operations.
January 24. Right femoral exposed under cocaine, by 11.40.
11.50. B.P., 130 mm. 11.52-12.00 bled 195 cc. 1-1.40 re-injected 260 cc.
1.42. B.P., 110 mm. 1.50 to 2.34. Dog off table.
2.37. B.P., 130 mm. 2.39-4.3, Bled 210 cc. 2.44, B.P. 60 mm.
3.38-4.0. Reinjecting 230 cc. 3.41 B.P., 110 mm.
3.54-4.01. Bled 200 cc. 4.03, B.P., 55 mm. 5.03, B.P., 70 mm.
5.05-5.09. Reinjecting 210 cc. 5.10, B.P., 110 mm. 5.42 B.P., 135 mm.
5.44-5.49. Bled 185 cc. 6.02 B.P., 50 mm.
6.36-6.40. Reinjecting 210 cc. 6.40 B.P., 105 mm.
6.46. Bled 40 cc. for analysis.
January 25. 1.20, Right femoral artery prepared again. 1.47, B.P., 135 mm.
1.51-5.6. Bled 205 cc. 1.57 B.P., 100 mm.
2.58. B.P., 110 mm. 2.59-3.02, Reinjecting 210 cc. 3.03, B.P., 135 mm.
3.28. B.P., 135 mm. 3.29-3.35, Bled 200 cc. 3.37, B.P., 105 mm.
4.24. B.P., 100 mm. 4.25-3.0, Reinjecting 200 cc. 4.31, B.P., 120 mm.
4.32-5.00. Dog off table. 5.04, B.P., 115 mm. 5.05-5.14, Bled 200 cc.
5.14. B.P., 60 mm. 6.05, B.P., 80 mm. 6.06-6.08, Reinjecting 200 cc.
6.09. B.P., 105 mm. 6.15, B.P., 95 mm. 6.16-2.0, Bled 135 cc.
6.21. B.P., 50 mm. 6.47, B.P., 75 mm. 6.48-6.51, Reinjecting 200 cc.
6.52. B.P., 105 mm.
January 26. 11.16-12.30. Preparation of vessels under cocaine.
12.35-4.0. Bled 180 cc. 12.41, B.P., 105 mm. 1.27, B.P., 100 mm.
1.28-1.33. Reinjecting. 1.33, B.P., 110 mm. 2.06 B.P., 120 mm.
2.07-1.1. Bled 190 cc. 2.12 B.P., 52 mm. 2.53, B.P., 65 mm.
2.54-3.03. Reinjecting 190 cc. 3.04 B.P., 105 mm.

3.20. B.P., 100 mm. 3.21-27, Bled 175 cc. 3.28, B.P., 50. Collapse. Restlessness, rapid breathing, defecation, urination, unconsciousness.

3.47. B.P., 47 mm. 3.49-53, Reinjectd 190 cc. 3.55, B.P., 95 mm. Dog recovers consciousness, breathes slowly, mucous membranes red again. 5.27, B.P., 110 mm. 5.28, Bled 75 cc. Again signs of uneasiness and rapid breathing.

5.34. B.P., 70 mm. 5.35, Reinjectd 80 cc. Dog all right again.

The above protocols show clearly the progressive effect of plasmapheresis in lowering the blood pressure and diminishing the resistance to collapse on bleeding.⁷ On the first day the pressure keeps up well. The original high reading of 208 mm. was probably due to excitement. The afternoon figures 135-140 are probably nearer to the normal value when all the blood is in the system. A fall of 40-50 mm. at each bleeding increases to a 75 mm. drop at the last, with a recovery of 20 mm. in 23 minutes waiting. On the third day the pressure is still good, but a big fall of 70 mm. is noticed at the second bleeding. The next fall touches the low level of 55 mm. but the system is still able to recover spontaneously, reaching 70 mm. in an hour's waiting. Reinjection brings it to 110 mm. which improves 25 mm. more in half an hour. The next bleeding though smaller, causes a drop of 85 mm. On the fourth day the pressures are good for the first two bleedings and the low levels after the last two are followed as before by spontaneous improvements of 20 to 25 mm. On the last day only the first bleeding is well sustained, the second causes a low drop with a small recovery (13 mm. in 40 minutes waiting) while after the third, which caused a marked collapse it is noticeable that instead of rising, the pressure falls slightly (50 to 47 mm.) while waiting for reinjection. The small amount bled for analytical purposes, now causes another fall to a low level and symptoms of threatened collapse.

From these and other observations it seems that any pressure lower than 80 mm. is a danger signal, though 50 mm. may often

⁷ See, however, page 147 for the effect of imported hirudin, which was certainly used in the latter part of this experiment. No record was kept of the date at which the inferior hirudin was substituted for that made in this laboratory.

be reached without collapse. Especially, if this low point is touched for the second time, and if on waiting no spontaneous rise is noticed, the danger of collapse is very great. By taking the blood pressure frequently, interrupting a bleeding if in doubt, a valuable indication of the course of the procedure is obtained, which with experience will probably greatly increase the chances of avoiding overbleeding.

4. LIMITS AND DANGERS OF PLASMAPHAERESIS

In the first paper on plasmapheresis by Abel, Rowntree and Turner it was shown that large quantities of blood, amounting in one case to more than twice the estimated volume of circulating blood could be withdrawn by this method in 1 day by repeated bleedings without apparent injury and as much as three volumes in the course of 3 days, and the hope was expressed that these limits could be still further extended.

The maximum total amount which can be withdrawn by plasmapheresis has been raised in the present investigation to over five volumes, removed in the course of 5 days. The animal survived more than 3 weeks and then only succumbed, as we believe, to accidental infection. In this respect the earlier expectations have been realized, and still further progress may be possible with care, in certain cases.

Although much time has been devoted to determining by a series of experiments on extensive and prolonged plasmapheresis the safe limits of the process an unfortunate occurrence has entirely vitiated this work. The imported hirudin which was used interchangeably with the extract of leach heads which we prepared ourselves, was found, contrary to the repeated testimony of earlier workers to be highly toxic, as is shown in the following paper by E. K. Marshall, Jr. This factor, which was not ascertained till the end of the present work is certainly accountable for many of the deaths observed and probably lowered the resistance of other dogs which succumbed eventually to overbleeding. As no record was kept as to which kind of hirudin was used in each experiment it is impossible at this time to

eliminate all the experiments in which the toxic hirudin was used. On the other hand the large amounts of blood which have been withdrawn in many cases with comparative impunity, even though a certain amount of the hirudin used is known to have been of the toxic kind, indicate the probability of still greater success where good hirudin alone is used. For this reason it has not been thought well to withhold the results obtained, leaving the reader to use his own judgment as to the numerous cases of early collapse.

Many deaths were caused by a purposeful attempt to ascertain the limits of permissible bleeding, both as to rate, per day and total quantity. When the bleeding appeared to be well sustained the rate or amount was increased with the result that many experiments were cut short by sudden collapse, which might have been saved by a more cautious procedure.

One point of importance may be mentioned in this connection. The danger of collapse appears to be greater where the bleeding is very rapid. In the experiments recorded in the earlier paper almost all the bleeding was done by suction, through a needle inserted in a superficial vein. This was usually a slow process, from 20 minutes to 1 hour being required in most cases to obtain the blood abstracted at one time. When an artery is cannulated no such delay is experienced, and it is often difficult, in the effort to avoid clots to make the time of bleeding longer than 3 or 4 minutes. This rapid lowering of blood volume we believe to be a contributing cause to the numerous fatalities observed in the present investigation. A distinct improvement in technic appears to have been introduced in experiment No. 14 where the blood was withdrawn in four portions of about $7\frac{1}{2}$ per cent of the blood volume with 5 minute intervals between each portion, the four being then centrifuged simultaneously.

Before the toxic character of much of the hirudin used had been discovered it was noticed that the deaths observed in this series of experiments were of two distinct types. Acute death on the table follows overbleeding at any stage of the procedure with all the usual symptoms of asphyxia of the vital centers

in the medulla and spinal cord. It is to be noticed that when working at all carefully this does not occur at the moment of drawing blood. The amount decided on is taken in apparent safety. The dog though weak seems not perceptibly worse to the casual eye than often before. There is some air hunger, however, indicating that the depleted arterial system can no longer properly ventilate the medulla and in about 10 to 20 minutes when the blood is usually in the centrifuge and not immediately available there is a sudden collapse, usually vomiting, urination and defecation after which the breathing suddenly stops. The heart still beats and prompt reinjection of blood (saline has been tried but seems not to help much) with artificial respiration brings the animal around. After a little time he may appear as well as ever.

The second type of death occurred sometime after the conclusion of plasmapheresis, either, in cases of severe depression, the same night, or after an interval of several days. As shown in the following paper the lesions which were noted at autopsy³ are exactly those produced by injections of toxic hirudin without plasmapheresis: the same lesions may be present also in a less degree in those that survive. Ecchymoses and haemorrhagic patches are found in many parts of the body, especially in the mucosa of the intestines. In one case stomach and intestines were found full of blood, in another, lined with bloody mucus, while in experiment No. 8 which survived 3 days of plasmapheresis with the removal of 231 per cent, severe haemorrhages from the rectum and bloody stools led us to chloroform the animal for humanitarian reasons. These phenomena resemble the endotheliolytic action of certain poisons as sepsin, snake venom, etc. It was noticeable that no trace of them, at least by external signs was seen in many other dogs bled as much or more, from which we have concluded that they are not attributable to the process of plasmapheresis. We conclude therefore, in view of the entire agreement in the above findings with

³ The condition found at autopsy in Experiment 4 of the first paper (*loc. cit.*, p. 635-6) may be due to a similar cause. This however was a nephrectomized animal.

those to be submitted in the following paper by E. K. Marshall, Jr., that in the second type of death, the symptoms and autopsy findings are attributable solely to the very toxic hirudin which has recently been on the market.

PROTOCOLS

As most of the vital points of each experiment have been given elsewhere, the protocols are here condensed greatly. With regards to the findings at autopsy, absence of mention of capillary damage (haemorrhagic patches, etc.) must not be taken as evidence that the death was not due to hirudin, as these were not looked for till after they were noticed in experiment No. 8. Probably several of the earlier deaths were due in part at least, to this cause.

Experiment 1. Male dog 14 kg.

December 14. 60 per cent drawn in three bleedings from veins with needle and suction; 290 cc. at 11.40, 305 cc. at 2.30-3.30, 40 cc. at 5 p.m.

December 15. Dog well. 141 per cent drawn in 4 bleedings from cannulated artery: 375 cc. at 10.31-33, 405 cc. at 11.45, 395 cc. at 12.50 and 300 cc. at 2.45. Dog collapsed about 2.55. Autopsy negative except liver and spleen very large and dark.

Experiment 2. Dog 151 kg. Arterial bleeding throughout.

December 21. 111 per cent in 3 bleedings: 495 cc. at 12.33, 390 cc. at 2.45 and 300 cc. at 4.05. Sewed up. 21 cc. for analysis at 5.15.

December 22. Dog somewhat depressed, but seems well. 75 per cent in 2 bleedings: 485 cc. at 10.57, 365 cc. at 1.05. Dog collapsed about 1.15: Died.

Experiment 3. Dog 11.9 kg. Ether anaesthesia; kymograph; arterial bleeding.

January 11. 11.15, Ether. 11.30 prepared both femoral arteries. 11.45, Prepared thoracic duct. Attempts to cannulate: discarded at 1.20. Bled 350 cc. (39 per cent) at 1.30, dangerous drop of pressure. Reinjectd 500 cc. at 2.31. Bled 350 cc. at 3.05-9. Reinjectd 3.47. Bled 300 cc. at 4.38. Pressure fell to 20 mm. Returned hastily, 67 cc. blood with 133 cc. Locke. B.P., 70. Reinjectd remainder corpuscles made up to 260 cc. at 5.35. Bled 190 cc. at 6.01. B.P., 20

mm. Injected 200 cc. Locke. B.P., 60 mm. Reinjectd 200 cc. at 6.45. Ether off. Wounds dressed. Gave chloretone 1.5 gram. Later morphine. Dog died in night.

Experiment 4. Dog 10.8 kg. Ether anaesthesia; kymograph; venous bleeding.

January 15. Ether at 2.15. Preparation of jugular, right and left femoral arteries by 2.40. B.P., 150 mm. Bled 40 cc. for analysis at 3.17, 375 cc. at 3.45. 4.21, B.P., 150 mm. Bled 20 cc. at 4.43. Reinjectd 410 cc. at 4.54. Bled 310 cc. at 5.29-48. Dog died in collapse 6.15.

Experiment 5. Young dog 7.15 kg. Ether anaesthesia; kymograph; venous bleeding.

January 18th. Ether at 12.05 cannulated jugular vein, femoral artery and femoral vein. 12.25-35. B.P., 1248, Bled 145 cc. 1.28, Bled 15 cc. 1.33, Reinjectd 160 cc. 1.45, Bled 100 cc. 2.25, Reinjectd 120 cc. 2.55, Bled 25 cc. Dog collapsed, pressure falling slowly. 3.01, Injected 150 cc. saline. 3.28, Reinjectd corpuscles 65 cc. 3.47, Injected 100 cc. of Locke with 1 gram glucose. 4.10, Bled 75 cc. 4.23, Injected 50 cc. saline. 4.25, B.P., 46 mm. 4.56, Reinjectd corpuscles 95 cc. 5.11, Bled 65 cc. 5.29, Injected 75 cc. saline. 5.48, Reinjectd corpuscles 70 cc. 6.07, Bled 40 cc. (?) Dog collapsed, died. Autopsy. Muscles and subcutaneous tissue oedematous. Meteorism.

Experiment 6. See p. 135 for protocol and tables for blood counts, analyses, etc.

Experiment 7. Dog 6.7 kg. on March 8. Reds 9.7 millions.

March 9. Reds 8.5 millions. Hgb. 115 per cent. Whites 16,000. T. 38°. 11.09, B.P., 165 mm. 11.10, Bled, 185 cc. (37 per cent). Dog restless, panting. 12.06, Blood returned. 12.17, Bled 170 cc. Dog better. 1.04, Blood back. 1.48, Dog vomits. 1.50, bled 160 cc. 2.10, Vomits. 2.41, Blood back. 2.46, B.P., 102 mm. 2.50 Hgb. 140 per cent. Reds 11.8 millions. Whites 12,600. Wt. 6.5 kg. T. 40°.

March 10. 8.30, T. 39. Hgb. 130 per cent. Reds 10 millions, Whites 50,000. 9.54, B.P., 125. 9.56, Bled 160 cc. (32 per cent). 10.52, Blood back. Dog drowsy. 11.00, Bled 170 cc. Blood returned. 12.06, Bled 195 cc. Collapse feared. Injected 75 cc. saline. 12.54, Blood back. 1.00, Hgb. 150 per cent. Reds 11.1, Whites 33,000. Wt. 6.7. T. 40°.

March 11. Dog seems very well but has not eaten. Wt. 6.4. T. 38 $\frac{3}{4}$ °. Hgb. 12.8 per cent. Reds, 8.5. Whites, 31,000. 10.40, Bled

170 cc. Lost three-fourths of this by accident. Made up by blood of other dog. 11.45, Returned part. 1.15. Returned rest. T. 39°.

March 12. Wt. 6.5. T. 38.5°. Hgb. 123 per cent. Reds 10 million, Whites, 25,000. 9.51, B.P., 150 mm. 9.52, Bled 190 cc. (38 per cent). 10.49, Blood back. 11.00, Bled 180 cc. (36 per cent). Blood returned. 12.00, Bled 165 cc. (33 per cent). 12.46, Blood back. Dog appears to be in good condition. 12.54, Bled 145 cc. (29 per cent). 12.55, B.P., 85 mm. 1.38, Blood back. 1.44, Bled 110 cc. (22 per cent). 1.45, B.P., 65 mm. Dog collapsed suddenly and died. Amount drawn in day 158 per cent.

Experiment 8. Dog. Wt. 7.52 kg. T. 38.3°. Hgb. 120 per cent. Reds 10.3. Whites 20,000.

March 15. 12.10, Bled 135 cc. (24 per cent). Dog somewhat restless until blood returned. 1.46, Bled 130 cc. 2.46, Blood back. 2.55, Bled 130 cc. 3.39, B.P., 115 mm. 3.42, Blood back. 3.45, B.P., 150 mm. 3.55, Hgb. 145 per cent. Reds, 10.6. Whites, 11,000. T. 39.5. Wt. 7.55 kg.

March 16. Wt. 7.62 kg. T. 38°. Hgb. 127 per cent. Reds, 10.2. Whites, 77,000. Dog in good condition. 9.49, B.P., 130 mm. 9.55, Bled 155 cc. (28 per cent). 10.45, Blood back. 11.00, Bled 147 cc. 12.05, Blood back. 12.10, Bled 145 cc. 1.10, Blood back. Condition good. B.P., 105 mm. T. 40°. Wt. 7.71 kg. Hgb. 130 per cent. Reds, 11.3. Whites, 27,000.

March 17. Wt. 7.39 kg. T. 38°. Hgb. 120 per cent. Reds, 9.5. Whites, 54,000. 11.27, Bled 154 cc. (27 per cent). 12.32, Blood back. 12.35, Bled 156 cc. 1.25, Blood back. Dog appears to be in good condition. 1.35, Bled 155 cc. (27 per cent). 1.40, B.P. 40 mm. Dog very uneasy. 2.10, Dog collapses, pupils dilated, unconscious, breathing stops. Injected 100 cc. saline, with artificial respiration followed quickly by return of corpuscles. Dog saved by close margin. 2.20, T. 38°. Blood drips from rectum. Dog has ulcerated gums which were not noticed till this morning.

March 18. Dog in poor condition. Blood in stools and on floor of cage. Urine normal. Wt. 7.15 kg. T. 37.5°. Hgb. 106 per cent. Reds, 7.7 million. Whites, 50,000.

March 19. Condition bad. Passes loose and bloody stools.

March 20. Same condition. Haemorrhage made blood counts meaningless, so chloroformed dog to save suffering. Autopsy, great congestion and hyperaemia of entire gastro-intestinal tract. Kidneys showed reddened areas suggesting fresh infarcts.

Experiment 9. Effect of gelatine. Dog. Wt. 6.27 kg. T. 38.7°. March 19.—10.30, Hgb. 110 per cent. Reds, 9.2. Whites, 12,000, 3.48, Operation begun. 4.19, B.P. 170 mm. 4.20, Bled 192 cc. (48 per cent). 4.23, B.P., 100 mm. Hgb. 105 per cent. 4.30, injected 120 cc. Locke's solution containing $2\frac{1}{2}$ per cent gelatine. 4.33, B.P. 200 mm. 5.27, B.P. 155 mm. 5.31 Blood returned, with Locke-gelatine as above (150 cc): same solution used throughout. 5.37, B.P., 180 mm. 5.38, Bled 207 cc. (46 per cent). 5.41, B.P., 70 mm. 5.54, B.P., 80 mm. 6.35, B.P., 100 mm. 6.40, Blood back: dog vomits. 6.43, B.P., 150 mm. 6.49, Bled 145 cc. (31 per cent). Hgb. 120 per cent. 5.53, B.P., 70 mm. 7.39, Blood returned. 7.40, B.P., 140 mm. T. 39.5°. Dog seems well: off table, urinates copiously. 10.15 p.m., Dog found unconscious, at intervals clonic convulsions, dyspnoea, later convulsions become more severe and tetanic, dog unconscious, screams at times. Twice resuscitated by artificial respiration after complete stoppage of breathing in severe convulsion, heart still beating, but finally dies at midnight.

Experiment 9 (bis). Control of gelatine experiment without plasma-phæresis. March 19. Dog. Bled 100 cc. Injected 250 cc. of Locke-gelatine (as above, but has stood overnight and fine turbidity which has passed through filter paper, had settled out) into saphenous vein.

March 20. Dog all right. Injected 120 cc. more of same Locke-gelatine. Dog perfectly well. No symptoms now or later. Observed for several days.

Experiments 10 and 11 were on hæmorrhage alone and are omitted here. *Experiment 12.* Effect of hæmorrhage on blood pressure and red count. See p. 142.

Experiment 14. Dog. Wt. 9.85 kg. T. 39°. Hgb. 115 per cent. Reds, 7.97 millions. Whites, 12,200. Arterial bleeding.

March 22. 11.40. B.P., 165 mm. 11.40–44. Bled 210 cc. (29 per cent). 12.44–49, Blood back. 220 cc. 12.56–59. Bled 220 cc. (30 per cent.). 1.46, Blood back. 225 cc. 2.23–32, Bled 215 cc. (5 cc. lost). 2.36, B.P., 48 mm. 3.19, B.P., 100 mm. 3.20, Blood back 2.40 cc. 3.25. B.P. 110 mm. 3.28 Hgb. 125 per cent. Reds, 10.7. Whites 6,000. Wt. 9.55. T. 39°. Dog in good condition. Vomits, defecates. Urine normal before and after operation.

March 23. Hgb. 130 per cent. Reds, 10.1. Whites, 23,400. Wt. 9.22. T. 38.7. 10.13, B.P., 100 mm. 10.14–20, Bled 155 cc. (21 per cent.) 10.22, B.P., 45 mm. Dog suddenly collapsed. Artificial respiration. Returned 60 cc. blood and 100 cc. saline. 10.30. B.P., 50. Dog

breathing. 10.35, Dog again stops breathing. Returned rest of blood and 100 cc. more saline. Dog taken off table, breathing, placed in cage. About 10.50 dies, respiratory failure. Autopsy at once. Stomach and intestines found full of blood. Small ecchymoses in mesentery and wall of gut. Mesentery vessels greatly dilated. Kidneys show dark fan shaped reddish areas on section. Lungs mottled with dark spots, apparently haemorrhagic, otherwise normal, as are liver and spleen.

Experiment 15. Dog Wt. 9.47 kg. T. 38.5°. Hgb. 97 per cent. Reds, 6.8. Slow arterial bleeding from femoral arteries. B.P. at start 180 mm.

March 23. 12.12-12.37, Bled 205 cc. (29 per cent) in four portions at about 5 minutes intervals. 1.25, Blood back. 2.43-3.09, Bled 210 cc. as before. 3.55, Blood back. 4.02, B.P., 110 mm. T. 40°. Dog in fine condition.

March 24. Dog ate all supper. Slightly depressed. Wt. 9.57. Hgb. 82 per cent. Reds, 7.1. T. 38.5°. B.P., at start 150 mm. 2.47-56, Bled as above 215 cc. 3.50, Blood back. 4.08-4.29, Bled 200 cc. 4.38, B.P., 80 mm. 5.20, Blood back. 5.26, B.P., 110 mm. T. 38°. Condition good. Urine normal.

March 25. Wt. 9.7 kg. T. 39°. Urine normal. B.P. at start 155 mm. 11.41-59. Blood as above 202 cc. Reds, 6.7 millions. 12.02, B.P., 145 mm. Restless. 12.55, Blood returned. 2.15-34, Bled 205 cc. 2.36, B.P., 100 mm. 3.15, Rapid breathing. 3.28, Blood back. 3.34, B.P., 120 mm. T. 40°. Hgb. 97 per cent. Reds 6.7.

March 26. Wt. 9.34. T. 38.5°. Reds, 5.8. Whites, 35,000. Urine normal. B.P., at start 145 mm. 11.46-12.07, Bled as above 195 cc. 12.09, B.P., 110 mm. 12.23, Respiration 32. Condition good. 12.55, Blood back. 2.15-2.20, Bled 140 cc. in three portions. 2.23, B.P., 110 mm., breathes very fast for a minute or two. 2.25, Bled 45 cc. Again breathing rapidly. 2.30, Cannula accidentally dislodged by animal's struggles 20 to 30 cc. blood lost. 3.15, Blood back. 3.50, T. 40°. Hgb. 82 per cent. Reds, 6.5. Whites, 47,000.

March 27. Dog seems very well, has eaten. Large yellow stool, no signs of blood. T. 38°. Wt. 9.15. Hgb. 65 per cent. Reds, 4.7 millions. Whites, 37,000. Urine normal.

March 29. Condition good. Slight oedema of one leg. T. 40°. Wt. 9.3. Hgb. 57 per cent. Reds, 4.3. Whites, 26,000.

March 31. Condition very good. T. 40°. Wt. 8.57. Hgb. 63 per cent. Reds, 4.3. Whites, 30,000. During Easter vacation dog developed a swelling, reported by janitor as close to insertion of penis. In night of April 5-6 wound perforated at this point and dog bled to death.

Experiment 16. Dog. Wt. 6.27 kg. Arterial bleeding with stopcock in cannula in femoral artery.

April 8. T. 38.5°. Hgb. 105 per cent. Operation began 12.00. 12.12-14, Bled 182 cc. (39 per cent). Blood back 12.55. 1.41-43, Bled 185 cc. (40 per cent). Blood back, 2.24. 2.48-50, Bled 146 cc. (32 per cent). Blood back, 3.29. 3.51-54, Bled 168 cc. (36 per cent). Blood back, 4.27. 5.09-14, Bled 128 cc. (27 per cent). Dog collapsed: 40 cc. saline injected; collapsed again at 5.33. Blood reinjected as soon as possible, partial consciousness returns. T. 38°. Dog off table: soon develops clonic convulsions, movements of fore and hind legs (swimming motion). Vomited; died shortly afterwards. Total amount withdrawn 174 per cent. Autopsy: Some small ecchymoses in intestinal walls and pericardium, dark haemorrhagic patches in lungs. Stomach and intestines opened showed bloody brownish fluid.

Experiment 17. Strong young bulldog. Wt. 8.2 kg.

April 9. Hgb. 90 per cent. Reds, 6.5 millions. Operation begun 10.29. 10.35, Bled 240 cc. (39 per cent). Blood back. 10.42, Bled 230 cc. (37 per cent). Blood back 11.24. At last bleeding dog became exceedingly restless, no signs of collapse, very vigorous. After return of blood still violent. Taken off table. Pants furiously and cries. Seems to have cardiac distress, but may be partly psychological. Calms down in about an hour.

April 10. Dog is somewhat depressed, but otherwise in good condition.

April 12. Condition good. Hgb. 85 per cent. Reds, 6.8. Whites, 10,000.

April 30. Condition excellent. Hgb. 107 per cent. Reds, 7.9. Wt. 9.3 kg.

Experiment 18. Puppy fox terrier. Wt. 3.5 kg.

April 9. 1.45, Hgb. 85 per cent. Reds, 7.0. Whites, 24,000. 1.51, Operation begun, cocaine: cannulated femoral. 2.11, Bled 117 cc. (45 per cent). Blood back, 2.42. 3.00, Bled 87 cc. (33 per cent). Blood back, 3.43. 3.45, Bled 64 cc. (24 per cent). Blood back 4.15. Total 102 per cent. No symptoms of collapse at any time. At end

of operation dog is very depressed, increasing up to 8 p.m. 12 mid-night, apparently unconscious, breathing labored. Dies in night. Autopsy: Patechiae in liver, lungs, etc. Interior of stomach and intestines lined with bloody mucus.

Experiment 19. Dog. 6.3 kg. Slow arterial bleeding from cannula into syringe.

April 20. 10.15. Hgb. 105 per cent. Reds, 8.3. Whites, 20,000. 11.00, Operation started. 11.14-24, Bled 193 cc. (41 per cent). 11.53, Blood back. 12.04-12, Bled 160 cc. (34 per cent). 12.38, Blood back. 1.48-55, Bled 138 cc. (29 per cent). 1.21, Blood back. Dog in good condition.

April 21. Hgb. 120 per cent. Reds, 8.8 millions.

April 29. Hgb. 95 per cent. Reds, 7.0.

Experiment 20. Dog. 8.7 kg. Venous bleeding.

April 21. 11.04. Operation begun. 11.12, Bled 235 cc. ($36\frac{1}{2}$ per cent). 11.48, Blood back. Dog appears perfectly well. 12.09, Bled 155 cc. (slowly) (24 per cent). Dog very depressed, vomits. 12.50, Defecates. Died in about 30 minutes before blood could be returned. Autopsy at once showed no apparent cause of death.

SUMMARY AND CONCLUSIONS

1. In suitable cases plasmapheresis may be carried even further than the limit reached in the first paper. From one dog over five times the volume of the blood has been withdrawn in five days. The dog survived several weeks and apparently only died from accidental infection.

2. In several other cases large quantities were removed, including one in which four blood volumes were withdrawn in four days, but owing to the toxicity of some of the hirudin supplied to us, the experiments were vitiated and proof could not be obtained of the safety of any particular technic.

3. Evidence of capillary poisoning was seen which was traced to a sample of hirudin used, as described in the following paper.

4. The blood pressure changes in plasmapheresis have been determined. The drop on bleeding was satisfactorily remedied on reinjection for a considerable time, but the low point reached progressively fell as the procedure was continued over two or three days. This may have been due in part to the toxicity

of the hirudin used at the end. Warning of danger may be obtained by keeping a careful watch on the pressure.

5. The number of both red and white cells has been followed in plasmapheresis. The former rise and the latter fall during a day's work, with a reverse change at night, in both cases. In long continued experiments the number of red cells at the beginning of each day slowly falls, and after the conclusion of the operation a moderate fall in the count appears to be a normal result; but further observations are needed on this point. No evidence of acute destruction of the corpuscles reinjected has been seen at any time.

6. Chemical analyses show a decrease of protein in the plasma to about one-third of its original value, caused by three days plasmapheresis, which was followed by a slight increase as the process continued. The urea and total non-protein nitrogen increased from the commencement.

7. A slight rise in temperature (about 1°) was observed during each day of plasmapheresis with a return to normal during the night.

THE TOXICITY OF CERTAIN HIRUDIN PREPARATIONS

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In the preceding article in this JOURNAL on plasmapheresis by Turner, Marshall and Lamson,¹ a series of fatalities are described from the practice of plasmapheresis. The results obtained were in discord with those previously published by Abel, Rowntree and Turner,² who found that large volumes of blood could be removed with the return of the corpuscles without harm to the animals. The latter authors used throughout the course of their work an aqueous extract of leech heads which had been coagulated at 82° and filtered. In their work on vivi-diffusion³ the same authors used mostly the above leech extract, although in some experiments the commercial preparation of hirudin manufactured by Sachse & Co. of Leipzig was used. In most of the work described in the preceding paper, the commercial hirudin was used. However, we had no reason at the time to ascribe the bad result to hirudin, or to think that any material difference existed between the two preparations.

Considerable literature has accumulated on the experimental use of hirudin. Kaposi⁴ has found 40 mgm. commercial hirudin non-toxic for a 2000 gram rabbit. Bodong,⁵ working with hirudin which he had prepared from leech heads, injected into rabbits 23, 50.5 and even 75 mgm. per kilo. He states that it has no effect upon the circulation, or respiration, and is in no way harmful to the animals. In Bodong's experiments the injections were made very slowly, from 4 to 8½ minutes being

¹ Turner, Marshall and Lamson: This Journal, 1915, vii, 129-155.

² Abel, Rowntree and Turner: This Journal, 1914, v, 625.

³ Abel, Rowntree and Turner: This Journal, 1914, v, 275.

⁴ Kaposi: Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1914, xiii, 386.

⁵ Bodong: Arch. f. exp. Path. u. Pharm., 1905, lii, 242.

required for the intravenous injection of 2 to 3 cc. of the solution, but von Hertzen and Öhman⁶ in a series of 12 experiments on rabbits found that the more rapid injection of 7 mgm. per kilo of hirudin (Sachse & Co.) caused a transitory decrease in blood pressure and pulse rate. Sievert,⁷ presumably using the commercial preparation, found injections of 50 and 100 mgm. in rabbits, caused symptoms for from 3 to 6 hours. Two injections of 100 mgm. each 4 hours apart killed a 2 kilo rabbit 25 minutes after the second injection. Cowie⁸ has given 35 doses of from 10 to 22 mgm. of hirudin (Sasche & Co.) to a rabbit over a period of 26 days. He states that at the end of this time the animal had gained in weight and was in every respect perfectly well. Abel, Rowntree and Turner⁹ in their vividiffusion experiments on dogs used very large quantities of hirudin which they prepared from leeches, and noted no evil effects on the surviving animals. Clinically hirudin injection has been used as a therapeutic measure in the treatment of eclampsia. Dienst¹⁰ gave 200 mgm. of the commercial preparation in 50 cc. saline, while Engelmann¹¹ has given from 200 to 300 mgm. in 17 cases.

It would appear, therefore, from the testimony of many experimenters, Sievert excepted, that hirudin when properly prepared may be used experimentally in amounts that suffice to render the blood non-coagulable without danger to the life of the animal, and clinically without untoward results.

In view of the untoward results obtained in many of the experiments on plasmapheresis described in the preceding paper, and especially in consideration of hemorrhages found at autopsy in several of the animals, it was thought advisable to make a study of the action of the commercial hirudin preparation on dogs. No experiments are reported of its use in dogs, although the large amount of work which has been carried on in this laboratory with the hirudin extract prepared from the leeches had con-

⁶ von Hertzen and Öhman: *Skan. Arch. f. Physiol.*, 1908, xx, 1.

⁷ Sievert: *Zeit. f. exp. Path. u. Therap.*, 1909, vii, 532.

⁸ Cowie: *Jour. Med. Research*, 1911, xxiv, 497.

⁹ Abel, Rowntree and Turner: *This Journal*, 1914, v, 275.

¹⁰ Dienst: *Zentralbl. f. Gynäk.*, 1909, xxxiii, 1697.

¹¹ Engelmann: *Zeit. f. Geburtsh. u. Gynäk.*, 1911, lxviii, 640; Engelmann and Stada: *München. Med. Woch.*, 1909, lvi, 2203.

vinced us of its non-toxicity. The first experiments showed a marked difference in the action of the commercial preparation on dogs. Twenty-one mgm. per kilo killed an animal after severe symptoms with the typical hemorrhagic picture which had been observed in some of the experiments on plasmapheresis, while 1.3 mgm. per kilo gave very marked symptoms of vomiting and diarrhoea. A series of experiments which are reported below were carried out with the preparation of Sachse & Co., of Leipzig, and a control series with the extract which we prepare from leeches. A few tests were also made with a crude unpurified aqueous extract of leech heads to see if the toxic body was present in the leech and had been removed in the purification of the extract. The effect of the different preparations on blood pressure has also been studied.

The commercial hirudin manufactured by Sachse & Co. of Leipzig was obtained at three different times;¹² in one gram tubes designated lot I, 0.1 gram tubes, lot II, and 1 gram tube, lot III. The solution prepared by dissolving this in 0.8 per cent sodium chloride was distinctly opalescent and contained visible particles. It was filtered before injection.¹³ All three preparations were extremely toxic for dogs, killing, when injected intravenously, in doses of about 20–25 mgm. per kilo, and giving very marked symptoms with doses of 1.3 and 3.1 mgm. per kilo. The following table gives a résumé of the experiments with this commercial product.

EXPERIMENT NO.	WEIGHT IN KILOS	DOSE MGM. PER KILO	REMARKS
1.....	4.70	21.3 lot I	Died in 7 hours
2.....	4.30	15.1 lot I	Very sick. Recovered
3.....	5.70	3.1 lot I	Sick. Recovered
4.....	7.51	1.3 lot I	Sick. Recovered
10.....	5.40	16.7 lot II	Sick. Died 3 days later
5.....	3.50	25.7 lot III	Died in 4 hours
13.....	4.60	25.2 lot III	Died

¹² Lot I was obtained on February 23, 1915, lot II, on May 8, 1915, and lot III, on May 12, 1915, through Bischoff & Co., of New York.

¹³ The contents of the tubes are supposed to be sterile. Sterile saline was used in preparing the solution and all apparatus used was sterilized.

The following protocols relating to dogs injected with this hirudin show its extreme toxicity. The noteworthy symptoms were severe vomiting and diarrhoea, with extreme collapse and bloody stools if a fatal dose be given. At autopsy the whole gastro-intestinal tract presented a condition of marked congestion and hemorrhage, and hemorrhagic spots were found in the lungs and pericardium and occasionally in the spleen.

Experiment 1. Dog H 1, weight 4.70 kilos.

May 6, 12 m. 100 mgm. of hirudin (Sachse & Co., lot I) dissolved in 50 cc. of 0.8 per cent saline were slowly injected intravenously. Dog appeared somewhat depressed, but otherwise normal immediately after injection.

2 p.m. Dog very sick. Has vomited and had severe diarrhoea. Mucous membranes of lips cold and pale. Animal vomited repeatedly and diarrhoea continued. At 6.30 p.m. apparently better.

8 p.m. Found dead.

Autopsy. Small amount of bloody fluid in peritoneal cavity, pleural cavity free of fluid. Stomach filled with bloody, frothy fluid. Mucosa of stomach and intestine congested and hemorrhagic. Lungs and pericardium show a few small hemorrhages.

Experiment 2. Dog H 2, weight 4.30 kilos.

May 6, 3.10 to 3.20 p.m. 65 mgm. of hirudin (Sachse & Co., lot I) dissolved in 35 cc. of 0.8 per cent saline injected intravenously. Dog made very sick. Vomited repeatedly, diarrhoea, lips cold and bloodless.

5.20 p.m. Still very sick.

8.00 p.m. Still vomiting; diarrhoea.

10.00 p.m. Better.

May 7. Dog eats heartily and seems normal.

May 8. Vomited during night. No diarrhoea.

May 10. Dog normal. Weight 4.25 kilos.

May 14. Vomited and had slight diarrhoea. Seems lively however.

May 27. Dog died of distemper.

Autopsy shows consolidation of about half of lung tissue. No hemorrhage found.

Experiment 3. Dog H 3, weight 5.70 kilos.

May 7, 3 p.m. Temperature 39°.

3.20–3.22 p.m. 18 mg. hirudin (Sachse & Co., lot I) dissolved in 9 cc. saline injected intravenously.

3.30 p.m. Dog seems depressed. Mucosa of lips pale.

3.33 p.m. Soft, black, tarry stool.

3.41 p.m. Vomited.

3.55 p.m. Vomited bile stained fluid.

4.00 p.m. Vomited and continues to retch.

4.07 p.m. Dog lies in cage, vomiting.

4.10 p.m. Temperature 38.5°.

4.40 p.m. Vomited.

4.55 p.m. Vomited.

5.45 p.m. Vomited. Temperature 40°.

7.30 p.m. Lying quietly in cage. Looks weak and sick.

9.00 p.m. Seems much improved.

May 8. Dog better, but has slight diarrhoea this morning.

May 10. Weight 5.35 kilos.

May 14. Vomited during night and had diarrhoea. Dog from now on seems to have recovered completely.

Experiment 4. Dog H 5, weight 7.51 kilos.

May 10. Injected 10 mgm. hirudin (Sachse & Co., lot II), dissolved in 5 cc. saline intravenously. A few minutes after injection, dog vomited. Vomited and very much depressed for next hour. No diarrhoea noticed. In 3 hours dog seemed pretty much normal.

Experiment 5. Dog H 7, weight 3.50 kilos.

May 15. 11.20 a.m. Temperature 37.5°.

11.24–11.28. Injected into leg vein, 90 mgm. hirudin (Sachse & Co., lot III), dissolved in 40 cc. saline.

11.28 a.m. Soft stool, seems depressed, mucosa pale.

12.00 Dog retches and vomits.

12.35 p.m. Lying down prostrated, lips pale and cold, extremities stone cold.

1.00 p.m. Temperature 34°. Dog continues in a state of extreme collapse.

2.10 p.m. Bloody stools. Practically unconscious, breathing slow and labored.

3.20 p.m. Has been unconscious for some time. Died.

Autopsy. Considerable blood in peritoneal cavity, pleural cavity free of fluid, a few hemorrhages in lungs and pericardium. Gastric mucosa and whole length of intestines shows extreme congestion and hemorrhages.

Microscope. *Intestine*, extreme congestion and submucous hemorrhages. *Liver*, cloudy swelling, central degeneration of cells, fatty infiltration, no necrosis. *Kidney*, cloudy swelling in convoluted tubules, intense congestion of glomeruli. *Spleen*, a few diffuse hemorrhages. *Adrenal*, appears normal.

Experiment 6. Dog H 8, weight 7.20 kilos.

May 15. Injected with 100 cc. same saline used in experiment 8. No symptoms whatever. Dog lively and perfectly normal.

The following protocols show that although toxic the commercial hirudin is not fatal to rabbits in much greater dosage than that which kills dogs. Whereas 21.3 mgm. per kilo proved fatal to a dog, rabbit H 10 survived a dose of 70.4 mgm. per kilo and when killed 24 hours later showed at autopsy no congestion and only a few hemorrhagic spots in the small intestine, lungs and pericardium. The anatomical picture was very mild compared with that of any of the dogs which came to autopsy.

Experiment 7. Rabbit H 4, weight 1.43 kilos.

May 7. Injected into the ear vein 30 mgm. hirudin (Sachse & Co., lot I) dissolved in 15 cc. of saline. Rabbit much depressed, has one or two soft stools and tries to vomit. Lies in corner of cage, refuses to eat for over 24 hours. After 2 days apparently quite normal.

Experiment 8. Rabbit H 9, weight 1.42 kilos.

May 17. Injected into ear vein 55 mgm. hirudin (Sachse & Co., lot III) dissolved in 17 cc. 0.8 per cent saline. Rabbit seems depressed and quiet compared with a control injected with saline. Next morning seems normal and eats heartily.

Experiment 9. Rabbit H 10, weight 1.42 kilos.

May 18, 11.30 a.m. Injected 100 mgm. hirudin (Sachse & Co., lot III) in 20 cc. saline. Rabbit had soft stool and appeared depressed.

May 19, 9.30 a.m. Chloroformed and autopsied at once. On opening peritoneal and pleural cavities no fluid found. A few sub-pericardial hemorrhages, hemorrhagic spots in lungs, gastric mucosa

clean, duodenum and upper third of intestines contain a few hemorrhagic spots. No congestion.

The severe collapse and great depression with the cold and bloodless mucosa of the dogs injected with large doses of the commercial hirudin suggested some immediate effect on blood pressure. The following three experiments, however, show that the only immediate effect on pressure is a slight transitory lowering, which is more pronounced the more rapidly the injection is given. This is directly comparable to the records attained with the non-toxic leech extract.

Experiment 10. Dog H 6, weight 5.40 kilos.

May 10. Femoral artery was exposed without anaesthesia and cannulated. Blood pressures were taken with manometer devised in this laboratory by Dr. B. B. Turner and described in the preceding paper.

5.00 p.m. B.P. 175 mm.

5.30 p.m. B.P. 150 mm.

5.41-5.43 p.m. Injected 90 mgm. of hirudin (Sachse & Co., lot II) into jugular vein.

6.06 p.m. B.P. 120 mm.

6.27 p.m. B.P. 140 mm.

Artery ligated and dog put in cage. Vomited and very much depressed. Mucosa of lips have been pale and cold for past half hour.

9.00 p.m. Dog lies in cage, very depressed.

10.15 p.m. Dog cannot be raised very easily. Seems very sick. No diarrhoea noticed.

May 11. Black, fluid stool this morning, otherwise appears normal.

May 12. Appears normal.

May 13. Dog found dead with large amount of blood in cage. Cannot state definitely whether it comes from artery or rectum.

Autopsy. Peritoneal and pleural cavities free of fluid. A few hemorrhagic patches on edge of lungs. Stomach filled with blood clots. Gastric mucosa hemorrhagic, small intestine slightly hemorrhagic, large intestine congested and covered with petechial hemorrhages.

*Microscope.*¹⁴ *Intestine*, marked congestion, and hemorrhages on outer part of mucous membrane. No leucocytic infiltration. *Liver*, slight amount of central degeneration of cells. A few globules of fat are seen in central cells. No necrosis. *Kidney*, epithelial cells show granular cytoplasm. In some places loss of staining power. No degenerative nuclei are seen. *Adrenal*, appears normal.

Experiment 11. Dog, weight 6.90 kilos.

May 10. This animal was treated exactly as Dog H 6 in experiment 6, except that he received 50 cc. of saline instead of hirudin solution. Blood pressure taken before and after the injection show practically no change, and dog appears normal in every respect.

Experiment 12. Dog H 11, weight 7.00 kilos.

May 27. Dog anaesthetized with ether and continuous tracing of blood pressure record taken from femoral artery.

4.06. B.P. 130 mm.

4.08-4.10½. Injected 30 mgm. hirudin (Sachse & Co., lot III) dissolved in 10 cc. saline. Blood pressure dropped during injection to 106 mm., but at end was 110 mm.

4.12. B.P. 134 mm.

4.12-4.14. Injected 21 mgm. hirudin (Sachse & Co., lot III) dissolved in 7 cc. saline. Pressure dropped to 120 mm. but before end of injection had risen to 130 mm.

4.22. B.P. 160 mm.

4.25. B.P. 150 mm.

4.30. Artery ligated.

4.55. Vomited.

During night vomited and had diarrhoea.

May 28. Seems depressed.

May 29. Seems normal, and showed no later effects.

Experiment 13. Dog H 16, weight 4.60 kilos.

June 3. Dog anaesthetised with ether, and continuous kymographic record of blood pressure from femoral artery taken.

3.35 p.m. B.P. 148 mm.

3.35-3.36 p.m. Injected 40 mgm. hirudin (Sachse & Co., lot III) in 10 cc. saline. Drop in pressure which continues even after end of injection.

¹⁴ For the preparation and study of the microscopic sections of this and other animals, I am indebted to Dr. D. M. Davis of the Pathological Laboratory of the Brady Clinic, Johns Hopkins Hospital.

3.36 p.m. B.P. 106 mm.

3.37 p.m. B.P. 80 mm.

3.39 p.m. B.P. 140 mm.

3.39-3.41½ p.m. Injected 40 mgm. hirudin (Sachse & Co., lot III) dissolved in 10 cc. saline. No change in pressure.

3.41½ p.m. B.P. 150 mm.

3.44 p.m. B.P. 144 mm.

3.44-3.45½ p.m. Injected 27 mgm. hirudin (Sachse & Co., lot III) dissolved in 9 cc. saline. Slight gradual drop.

3.45½ p.m. B.P. 110 mm.

3.49 p.m. B.P. 130 mm.

Dog after first injection had pale and bloodless lips. On coming out of ether, seems fairly well, but soon lay down in cage. Very much depressed. Vomited and had diarrhoea during night. Next morning found dead.

Autopsy: Peritoneal cavity filled with blood. Pleural cavity free of fluid. Large hemorrhagic spots in lungs and pericardium. Stomach and intestines very markedly congested and hemorrhagic. Liver and kidney look congested. Hemorrhages in spleen.

EXPERIMENTS WITH AN AQUEOUS EXTRACT OF LEECH HEADS

The leech extract containing the hirudin used in the following experiments was prepared as already described by Abel, Rowntree and Turner.¹⁵ One lot was prepared from 150 leech heads, and after filtering, the crude extract measured 120 cc. A portion of this was reserved for injection and is designated lot C. The remainder was coagulated at 82-85°, and filtered from the precipitated protein. This is designated lot A. A second extract from 100 leech heads on filtering measured 100 cc. A portion of this was reserved for injection (lot D) and the remainder coagulated and filtered (lot B). Estimated by the clotting test, and assuming that 1 mgm. of commercial hirudin will keep 5 cc. of blood from coagulating for forty-eight hours,¹⁶ lot A contained 5 mgm. per cc.; lot B, 5 mgm.; lot C, 10 mgm., and lot D, 6½ mgm.

¹⁵ Abel, Rowntree and Turner: loc. cit.

¹⁶ Franz: Arch. f. exp. Path. u. Pharm., 1902, xlix, 342.

The experiments reported below show conclusively that the toxicity of the commercial preparation of hirudin is not due to hirudin itself nor to any constituent of the leech heads extracted by water. Two or three times the dose (calculated as hirudin) of the commercial preparation which was fatal to dogs has been given *without any symptoms* except a slight depression following the injection. The blood pressure records show that the leech extract, whether the protein has been removed or not, produces a transitory fall in blood pressure, which may explain the temporary depression. Furthermore, Dog H 15, which was killed and autopsied 24 hours after an injection of a large dose of hirudin, showed perfectly normal organs with no sign of hemorrhages anywhere.

The standardization was performed as follows: varying amounts of hirudin solution were placed in test tubes and 2 cc. of dog's blood allowed to flow into each. The tubes were observed at the end of 24 and 48 hours and the smallest amount of hirudin necessary to render the blood incoagulable was noted. From this the amount of solution necessary for 5 cc. of blood was calculated.

Experiment 14. Dog H 12, weight 4.45 kilos.

May 24. 3.21-3.23. Injected 45 cc. (225 mgm.) hirudin solution (lot A) into leg vein.

3.25. Mucosa of lips pale, dog appears slightly depressed.

3.35. Dog lively. Mucosa red. Seems normal. No diarrhoea, no vomiting. Perfectly normal rest of afternoon. Dog has been perfectly normal to time of writing and weighed on May 31st 4.80 kilos and on June 8th weighed 5.15 kilos and was in perfect condition.

Experiment 15. Dog H 15, weight 8.35 kilos.

Animal anaesthetised with ether and continuous kymographic record of blood pressure taken at femoral artery.

May 31. 3.41 p.m. B.P. 100 mm.

3.42-3.43. Injected 10 cc. (50 mgm.) hirudin solution (lot B) intravenously. Blood pressure dropped during injection to 60 mm. but 1 minute after end of injection had risen to 95 mm.

3.47. B.P. 110 mm.

3.47-3.49. Injected 10 cc. (50 mgm.) hirudin (lot B).

3.49. B.P. 80 mm.

3.50. B.P. 100 mm.

3.59. B.P. 90 mm.

3.59-4.03. Injected 10 cc. (50 mgm.) hirudin solution (lot B).

4.03. B.P. 70 mm.

4.05. B.P. 92 mm.

Remains at about this level for 10-15 minutes. Record discontinued. No vomiting or diarrhoea. Dog perfectly normal after coming out of ether.

June 1. Dog normal, eats well and very lively.

4 p.m. Killed and autopsied at once. Tissues all look normal and no hemorrhages can be found anywhere.

Microscope: Liver and kidney appear normal.

Experiment 16. Dog H 13, weight 7.27 kilos.

May 24. 12.22-23½. Injected 25 cc. (250 mgm.) hirudin solution (lot C) intravenously.

12.25. Mucosa of lips pale, dog slightly depressed.

12.35. Mucosa still pale but dog is fairly lively.

12.40. Dog quite lively, apparently entirely normal. Mucosa of lips has better color.

1.00. Dog normal.

Dog observed for rest of afternoon and apparently quite normal.

May 25. Dog normal, no vomiting or diarrhoea during night.

Experiment 17. Dog H 14, weight 5.00 kilos.

May 31. Dog anaesthetised with ether, and continuous kymographic record of blood pressure from femoral artery taken.

4.45. B.P. 114 mm.

4.45-4.46½. Injected 10 cc. (65 mgm.) hirudin solution (lot D).

4.46½. B.P. 74 mm.

4.47. B.P. 60 mm.

4.48. B.P. 112 mm.

4.55. B.P. 100 mm.

4.55-4.56. Injected 5 cc. (32½ mgm.) hirudin solution (lot D.)

4.56. B.P. 80 mm.

4.56½. B.P. 68 mm.

4.58. B.P. 100 mm.

Record discontinued after about 10 minutes, no further noticeable change being observed. Artery ligated. Dog vomited during night, but no diarrhoea. (Vomiting probably due to ether.) In next few days dog observed and appeared normal.

June 4. Dog developed distemper and died.

Autopsy. Showed broncho-pneumonia with consolidation of about one-half lung tissue. No hemorrhages found anywhere.

SUMMARY

The preparations of the commercial hirudin of Sachse & Co., which we have examined have proven to be very toxic for dogs, but much less toxic for rabbits. Doses of from 20 to 25 mgm. per kilo proved fatal to dogs with symptoms of severe bloody diarrhoea and continuous vomiting. Smaller doses (even 1.3 mgm. per kilo) gave definite symptoms of depression, vomiting and diarrhoea. At autopsy, the dogs showed a very marked congestion and hemorrhagic condition of the intestinal tract with hemorrhagic patches in the lungs and pericardium and occasionally in the spleen. Examination of sections shows definite changes in the liver and kidney. The toxic action has been shown not to be due to the hirudin of the preparation nor to any substance of the leech heads extracted by distilled water. The effects on rabbits very much resemble those obtained by Sievert, who probably had a similarly toxic preparation. While the picture at autopsy very strongly suggests death from some product of putrefaction, and resembles very closely that described for sepsin,¹⁷ the character of the toxic principle has not been further investigated.

The leech extract obtained by extraction with water and coagulation of the proteins at 82°-85° is non-toxic in quantities that render the circulating blood non-coagulable and is without apparent effect upon the subsequent health of the animal. This confirms the conclusions of Abel, Rowntree and Turner concerning hirudin in their vividiffusion experiments. The blood pressure experiments confirm on dogs the observations of von Hertzen and Öhman on rabbits that the injection of hirudin causes a transitory fall in pressure. The fact that Bodong observed no change in blood pressure in rabbits on injection of hirudin is probably explained by the extreme slowness of his injections.

¹⁷ Bergmann and Schmiedeberg: *Cent. f. med. Wiss.*, 1868, vi, 497; Levy: *Arch. f. exp. Path. u. Pharm.*, 1894, xxxiv, 342; Faust: *ibid.*, 1904, li, 248

THE ROLE OF THE LIVER IN ACUTE POLYCYTHAEMIA: A MECHANISM FOR THE REGULATION OF THE RED CORPUSCLE CONTENT OF THE BLOOD

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I. INTRODUCTION

The increase of the number of red cells per unit volume of blood is a subject which has been of great interest to many investigators in the past. At present on account of the immense amount of work being done on the respiration and blood, this subject is receiving especial attention.

It has long been known that residence at high altitudes would increase the red count¹ in both man and animals. But experiments in which this method of increasing the number of red cells was used required much time, labor, and expense. Besides this the changes brought about were gradual, allowing complications to enter into the experiments.

Fortunately I was able to find from experiments of my own, and by a review of the literature, methods by which the number of red cells per unit volume of blood could be greatly increased in a few minutes. Also these methods were applicable to experi-

¹ The terms, number of red cells, red count, etc., will be used for the sake of brevity, in place of the more exact term, number of red cells per unit volume of blood.

ments carried out on animals under ether, and consequently allowed perfect control of experimental conditions.

Since the work of Cohnstein and Zuntz (1) in 1888 a great deal of work has been done in collecting accurate data concerning changes in the number of red cells, and in the percentage haemoglobin content of the blood, in various conditions. But there has been very little advance in our knowledge of how this increase in number of red cells takes place.

Before going further, let us see from purely logical reasoning how many ways there are in which the number of red corpuscles per unit volume of blood may be increased.

If we consider the circulation to be a closed system, the number of red cells may be increased by the following methods only.

- (1) A decrease in plasma volume.
- (2) An actual increase in the number of red cells by—
 - (a) A new production of red cells.
 - (b) A division of red cells.

(3) If the red cells are unequally distributed throughout the circulation, being packed away in certain places in greater concentration than in the general circulating blood, a stirring up of these, causing a more even distribution, will relatively increase the red count.

- (4) A combination of any of these factors.

Keeping in mind that any increase in the number of red cells must take place according to one of these methods, or a combination of them, let us pass over for the present which of these methods actually does take place in the body, and take up first the factors capable of increasing the red count.

II. FACTORS CAPABLE OF INCREASING THE RED COUNT

(1) *Lung emboli*

(a) *Corpuscles hardened with formaldehyde.* When injecting intravenously a few cubic centimeters of pig's corpuscles hardened with formaldehyde, into a dog, with the intention of recovering these, and thus possibly obtaining an index of the blood volume, it was surprising to find that the red count in the dog

rose from 7,590,000 to 11,096,000 in forty-five minutes. This rise was entirely out of proportion to the number of red cells injected and furthermore none of the pig's corpuscles could be recovered.

Repetition of this experiment showed that the number of red cells per cubic millimeter in the dog could be increased one or two millions in fifteen minutes or less by the injection of about 20 cc. of a suspension of these hardened corpuscles.

Experiment 1, November 18, 1914. A suspension of pig's corpuscles hardened with formaldehyde in salt solution, containing 2,371,000 corpuscles per cubic mm., was used. 20 ccm. of this suspension was injected intravenously into an 8 kilo dog.

Reds, 7,590,000 before injection.

Reds, 11,096,000, 45 minutes after injection.

Reds, 10,184,000, 75 minutes after injection.

November 19, same dog perfectly well, urine clear. Albumin 0.

Hgb. 105 per cent. Reds, 8,504,000.

Twenty ccm. of the same hardened corpuscles were injected intravenously. The dog struggled, frothed at the mouth, panted very fast, vomited, and had a stool, then became quiet, and appeared afraid to move. In one half an hour was apparently perfectly well again.

Hgb. 123 per cent. Reds, 10,176,000, 13 minutes after injection.

Hgb. 105 per cent. Reds, 7,340,000, 2 hours 10 minutes after injection.

November 20. Dog well; urine normal.

Experiment 2, November 19, 1914. Dog, 12.3 kilo.

Hgb. 100 per cent. Reds, 7,312,000.

Injected 25 cc. of the same hardened corpuscles.

Hgb. 120 per cent. Reds, 11,024,000 15 minutes after the injection.

Experiment 3, November 23, 1914. Dog, 6.1 kilo.

Hgb. 106 per cent. Reds, 7,024,000.

Injected 20 ccm. of less hardened corpuscles.

Hgb. 118 per cent. Reds, 7,584,000 15 minutes after injection.

Hgb. 118 per cent. Reds, 8,968,000 1 hour after injection.

Hgb. 110 per cent. Reds, 8,640,000 1 hour 45 minutes after injection.

Experiment 4, December 4, 1914. Dog, 5.8 kilo.

Reds, 6,736,000, before injection.

Reds, 6,872,000, 15 minutes after injection of pig's corpuscles treated overnight with a 4 per cent formaldehyde solution.

Reds, 6,816,000. One hour after injection.

This result was peculiarly striking, as it is so generally supposed that the number of red cells per unit volume of blood is a fairly fixed quantity, subject to gradual changes only. A hasty search in various clinical books showed this to be the general impression to be found there.

Polycythaemia at high altitudes, in chronic pulmonary conditions, certain congenital heart conditions, in patients desiccated by loss of fluid, and in one special disease, chronic polycythaemia with enlarged spleen and cyanosis, is very generally spoken of, but there is practically no mention of acute changes in the red count.

I went on then to find out if possible the reason for this sudden increase in the number of red cells after the injection of corpuscles hardened with formaldehyde.

It appeared that the degree to which the corpuscles were hardened had a marked influence on the amount of change in the corpuscle count, as shown in experiments 3 and 4. Besides this the dogs after the injection showed great dyspnoea, and signs of cardiac distress for some minutes after the injection. As these corpuscles were known to be somewhat agglutinated, it was thought that perhaps the hardened corpuscles acted as emboli in the lungs, causing dyspnoea and cardiac pain.

(b) *The injection of lycopodium, and of oil.* Substitution of an inert powder, in this case lycopodium, or of oil, for the corpuscles hardened with formaldehyde, as a means of producing lung emboli, gave a similar and very rapid rise in the number of red cells.

Experiment 5, December 9, 1914. Dog, 6.1 kilo.

Ether. Blood pressure apparatus attached to right femoral artery.

Hgb. 105 per cent. Reds, 8,552,000, before injection of lycopodium.

Hgb. 120 per cent. Reds, 11,464,000, 4 minutes after injection.

Experiment 6, December 11, 1914. Dog.

Etherized. Blood pressure apparatus attached to femoral artery.

Reds, 5,560,000.

6 ccm. of a fine emulsion of olive oil injected intravenously.

Three minutes later 8 ccm. and then 10 ccm. of plain olive oil.

Reds, 7,152,000, 10 minutes after beginning of injection.

Reds, 7,104,000, 25 minutes after first injection.

Four ccm. of thick machine oil was then injected.

Reds, 8,736,000, 40 minutes after first injection.

Reds, 9,504,000, 85 minutes after first injection.²

That lycopodium powder and oil both cause lung emboli when intravenously injected is certain from finding granules in the lungs, from the literature on emboli produced by the injection of oil (2), and from the following experiments which gave the typical picture of obstruction to the pulmonary circulation.

During the experiments on the effect of the injection of lycopodium powder and of oil, the chest was opened under artificial respiration, and the heart observed.

A short time after the injection, the right heart could be seen to beat more forcibly, then to gradually dilate, until it ceased to beat. On feeling below the right heart, the left ventricle was felt beating strongly but greatly contracted.

Injection of substances capable of causing lung emboli gave rise to dyspnoea, pain, fear, cardiac dilatation, and cyanosis, and to an increase in the red count. The question then arose as to which of these was the cause of the polycythaemia. Pain and fear in these experiments were ruled out by the use of ether. They will be spoken of in later experiments. The blood pressure showed no marked change, after the injection of either lycopodium or of oil, except toward the end where it gradually fell. Further experiments will show that in conditions where the heart has failed markedly there is not necessarily an increase in the red count. That cardiac failure is not the only factor capable of producing a polycythaemia is easily shown in experiments where the circulation is practically unchanged, and yet where there is a very marked increase in the number of red corpuscles.

² Injection of these substances is followed by very variable results, because too little will give no increase in the red count, and too much will cause death.

(2) *Asphyxia*

In these experiments there is undoubtedly a certain degree of asphyxia, the dogs appear cyanotic and the venous blood drawn appears very dark. Experiments carried out by Kuhn (3) in which he has caused asphyxia in man by a mask devised by himself show an increase of one million in the red count in one hour. This may be easily accomplished in animals by putting them under ether, and holding their noses, as is seen in the following experiments.

Experiment 7, December 12, 1914. Dog.

Ether. Reds, 9,016,000.

Reds, 10,864,000 after 18 minutes asphyxia.

Experiment 8, January 9, 1915. Dog.

Ether. Reds, 7,592,000.

Reds, 8,408,000 after 20 minutes asphyxia.

The opinion that asphyxia is responsible for this polycythaemia is further strengthened by the fact that oxygen will reduce the number of red cells markedly in certain of these conditions as shown by Kuhn (4), Croon (5), Bence (6).

As we know that residence at high altitudes, cardiac complications with cyanosis, chronic lung conditions, and this peculiar disease mentioned before, with chronic cyanosis, enlarged spleen, and polycythaemia (7), all indicate a form of asphyxia, they form a group of conditions as causative factors of polycythaemia, into which lung emboli will very well fit.

(3) *The effect of haemorrhage*

There is another method of producing a temporary asphyxia, and that is by the sudden removal of large volumes of blood. If the assumption that asphyxia in any form will increase the number of red cells is correct, sudden haemorrhage should be followed by an attempt on the part of the body to regain the normal red corpuscles content of the blood.

As is well known (8), acute haemorrhage lowers the red count, but not very markedly for some time after the blood is drawn.

An example of this is given in experiment 9 where after 74 per cent of the calculated blood in the body was drawn the count fell only 36 per cent, and the haemoglobin 20 per cent.

Experiment 9, March 20, 1915. Dog. Weight 8.3 kilo. Blood volume calculated as 7.5 per cent of body weight = 622 ccm. 10.05, ether. Blood pressure recorded with mercury manometer attached to right femoral. Blood drawn from a cannulated left femoral artery. Counts done from this blood after about 30 ccm. had run out of the cannula (for washing).

TIME	BLOOD DRAWN	HAEMOGLO- BIN	REDS IN MILLIONS	B. P. IN MM. OF HG.	TOTAL PER CENT BLED	TOTAL PER CENT DECREASE IN HGB.	TOTAL PER CENT DECREASE IN RED COUNT
	cc.	per cent					
10.06.		120	10.68	142			
10.26.	250						
10.33.		130	8.89	88	40	10.0	16.7
11.30.		117	9.05	140	40	3.0	15.1
11.45.	110			94	58		
11.52.	98			26	74		
11.57.		105	8.28	26	74	15.0	23.4
1.00.		100	7.30	20	74	20.0	26.3

A discussion of the changes taking place here would involve more time than the space here allows, but it is evident that the body does not immediately replace the loss of blood by a corresponding increase in fluid, to regain the normal volume, as in this case the red count would fall much more markedly.

That after haemorrhage the body may increase the number of red corpuscles per unit volume of blood above that found after the blood is drawn is shown by the fact that *occasionally* a short time after a haemorrhage the count is higher than immediately after the blood is lost. This is shown in the following experiment.

Experiment 10, January 11, 1915. Dog. 11.9 kilo. Ether.

1.30. Reds, 11,808,000.

350 ccm. blood drawn from the femoral artery.

Reds, 9,712,000, at end of bleeding.

2.22. Reds, 11,368,000.

3.05. After the return of the corpuscles drawn, which had been washed and suspended in normal salt, diluted to the volume of blood drawn.

Reds, 13,760,000.

(4) *The effect of plasmapheresis*

Furthermore, return of the corpuscles drawn, only after washing and suspending in salt solution to make up the volume bled, as has been done by Abel, Rowntree, and Turner (9), causes a marked increase in the corpuscle content of the blood, and indicates that after haemorrhage there is a sudden attempt by the body to regain the normal red corpuscle content of the blood.

Further examples of this are given in the following experiments.

Experiment 11, January 18. Dog. 7.15 kilo. Calculated blood vol. = 532 ccm.

	WEIGHT	HGB.	REDS	WHITES	VOL. BLOOD DRAWN	VOL. BLOOD RETURNED
March 8.	7.0		9,744,000			
March 9 {a.m.	6.7	115	8,480,000	16,000	185
					170	170
p.m.	6.5	140	10,760,000	12,600	160
					—	180
					515	535
March 10 {a.m.		130	10,000,000	50,000	160
					170	170
p.m.	6.69	150	11,064,000	33,000	195
					—	305
					525	635

Experiment 12.

	TIME	REDS	VOL. BLOOD DRAWN	VOL. BLOOD RETURNED
January 22.....	11.40	8,480,000	250	250
	2.24	9,240,000	250	250
	4.14	9,744,000	220	220
	6.50	8,528,000	720	720
January 23.....	11.28	7,152,000	200	200
	2.21	6,272,000	125	150
			170	170
			495	520
January 24.....	11.53	6,000,000	195	260
	3.54	5,960,000	200	210
	5.44	7,392,000	185	210
	6.46	7,576,000	40	—
			620	680
January 25.....	1.51	5,216,000	205	210
	3.29	5,440,000	200	200
	5.05	6,304,000	200	200
	5.16	6,952,000	135	200
			740	810
January 26.....	12.35	5,344,000	180	180
	2.07	6,048,000	190	190
	3.21	6,544,000	175	190
	5.28	7,960,000	45	45
			590	605
January 27.....	11.08	5,176,000	—	—

For a further study of blood changes in plasmapheresis, see Turner, Marshall and Lamson, note 10.

(5) *The general neglect of the importance of changes in the red count*

On careful examination of the literature, it will be found that a great deal of work has been done on the changes in the number of red corpuscles, and that several methods are known by which this number may rapidly be varied. It is curious, however, with the amount of work which has been done along these lines, that this *rapidity* of change in the number of red cells has been so generally overlooked, both in the laboratory and in the clinic.

In laboratory experiments one finds many instances where the total solids of the blood have been estimated by the difference in weight before and after drying. A variation in the total solids found by this method was looked upon as a change in the blood volume, no account being taken of the possibility of a change in the number of red cells, which on account of their higher specific gravity would increase the total solids.

In the clinic it is quite customary to do a rough haemoglobin test, and if this is 80 per cent or above, to omit a red count, the object being to discover all cases of anaemia, but apparently considering that an increased haemoglobin content is of little consequence. No attention was ever paid to a haemoglobin content of 110–115 per cent while a red, white, and differential count was always done on cases having a haemoglobin content below 80 per cent.

(6) *Review of the factors capable of increasing the red count*

Historically the first physiological condition in which a marked increase in the number of red cells was observed occurred in counts made at high altitudes. In pathological conditions, chronic cardiac cases with marked cyanosis, and congenital heart cases in which cyanosis was a prominent sign, were among the first in which the red cells were found to be increased.

Since Viault (11) first counted the red corpuscles of men and animals living at high altitudes, and Gaule carried on similar experiments in balloons (12) many data have been gathered concerning the changes in the number of red corpuscles under

these conditions (13). The results of these experiments all show that the red cells are increased in number, and that the haemoglobin is also increased, but not necessarily in proportion to the red count (14).

Experimentally, atmospheric pressures corresponding to those of high altitudes have been produced in chambers, and counts done on both animals and men kept in these chambers. The increase in number of red cells under these conditions is similar to changes observed at altitudes of the corresponding pressure (15).

To show that this increase in the number of red cells is due to a reduced tension of oxygen in the inspired air, and not to some secondary effect of the general decrease in atmospheric pressure on the body, animals and men have been placed in chambers in which the atmospheric pressure was kept normal, but the partial tension of the oxygen reduced to that of high altitudes (16). Under these conditions the red cells were increased in number as before.

In all of the above conditions the amount of oxygen per unit volume of inspired air is decreased, and thus the oxygen supply to the body will be reduced, unless compensated for by changes in the circulation, respiration, or blood.

If instead of a reduced oxygen content of the inspired air, the oxygen capacity of the blood is diminished, as occurs in cases of carbon monoxide poisoning in man, or experimental poisoning in animals, it has been shown that there is an increase in the number of red cells per unit volume of blood (17).

Furthermore if the oxygen content of the inspired air remains normal, but on account of experimental or pathological conditions the amount of oxygen reaching the blood per unit of time is decreased, as in pathological conditions, or in experimental stenosis of the air passages (3), as carried out in the above experiments, the red cells are again increased in number.

And finally where there is a greater consumption of oxygen, calling for a greater supply, the number of red cells is again found to be increased.

We see then that a reduction of atmospheric pressure, of the

partial tension of the oxygen in the inspired air, interference with the oxygen supply to the lungs, or with the blood supply to the alveoli, reduction of the oxygen capacity of the blood, or an increased consumption of oxygen by the body, may cause a polycythaemia.

Or in other words asphyxia of any type will act as a stimulus to increase the red corpuscle content of the blood.

Lack of oxygen is of course not the only factor in many of these conditions, an increased carbon dioxide content of the blood besides an increased acidity being present in several of them. These factors are however not present in all of the above conditions, and may be looked upon as secondary to the lack of oxygen.

We have then one very effectual stimulus, namely lack of oxygen, which may bring about an increase in the number of red cells per unit volume of blood, and it is one which may occur under many physiological conditions, as we have seen, namely high altitudes, exercise, etc.

If now we run over the conditions and substances which have been found by different investigators to increase the number of red cells per unit volume of blood, we will find that they are very many.

Tuberculin (18), toluylendiamine (19), thorium X (20), sulphur (21), strophanthin (22), splenic extract (23), sodium nitrite (24), serum from anaemic animals (25), radium chloride (26), the premenstrual state (27), pituitrin (28), pilocarpine (29), phosphorus (30), opium (31), nicotine (32), massage (33), liver disease (34), exercise (35), epinephrin (36), enzyme action (37), digitalis (38), cirrhosis of the liver (39), certain clinical conditions (40), carbon monoxide (41), caffeine (42), injections of blood (43), bleeding (44), bile (45), arsenic (46), adrenal extirpation (47), stimulation of the cut cervical cord (48), plasmapheresis (49), lung emboli (page 171), asphyxia (page 175).

(7) *Emotional stimuli*

Besides these numerous ways of producing polycythaemia I have found a stimulus of a still different type, namely an emo-

tional stimulus. In the large number of experiments which were carried out in this work (some 150) an animal was occasionally found with a very high initial count.

I could at first find no reason for this high count. The animals received the same diet, and were given an unlimited water allowance. They were kept at the same temperature, and no single factor could be found to account for the higher count in some than in others.

Excitable dogs which had been kept in the room for some time before being etherized, and which had played about a great deal, undergoing violent physical exertion, appeared at first to be the ones in which the counts were high. This seemed to agree with the finding of (50) Zuntz and Schumberg, Willebrand, Hawk, Tornow, Mitchell, Jones, Schneider and Havens and Boothby, who showed that exercise will cause the red count to increase. Others however which took the same amount of exercise had normal counts, and still others which were exceptionally quiet had higher counts than any.

Finding in the literature that epinephrin causes a very marked polycythaemia (51) when injected into animals, and connecting this fact with the experiments of Cannon (52) which showed that emotional stimuli such as fright, rage or excitement, cause an increased output of epinephrin, it occurred to me that the excitement of bringing these animals into new surroundings, putting them under ether, etc., might have caused the increased count found in some of them.

To confirm this idea, cats were tied down without ether, and a count taken as soon as possible from the jugular vein. The cat was then frightened for a minute or two by allowing a dog to bark at her. Counts done from seven to ten minutes after this showed a very great increase in the number of red cells.

It is also of interest to note that when the cats were tied down without ether, although there was practically no struggling, the initial counts were exceptionally high.

The results of these experiments are given below.

Experiment 13, March 5, 1915. Cat.

Tied on animal board without ether.

Reds, 10,720,000.

Dog was allowed to bark at the cat, which responded by spitting and jumping, and great changes in the size of the pupils.

Reds, 14,920,000, five minutes after the dog was brought in.

Experiment 14, March 8, 1915. Cat.

Same procedure as in experiment 13.

Reds, 15,680,000. Before fright.

Reds, 16,776,000. Seven minutes after fright.

Experiment 15, May 5, 1915. Cat.

Same procedure as above.

Reds, 11,576,000, before fright.

Reds, 12,216,000, five minutes after allowing a dog to bark at the cat for one-half minute.

Reds, 14,464,000. Seven minutes later.

The rapidity with which this increase takes place and the extremely high counts obtained in these experiments are very striking. It is of interest to note that these results bear out the statement of the physiologists, that a physiological stimulus is always more effective than any artificial one.

On reviewing the results of all the experiments carried out in this work, it was seen that in many animals where counts were taken immediately after putting the animals under ether, with the usual struggle and excitement of this, the count was higher than normal, but fell gradually as the anaesthesia was carried on.³

There is then evidence in both man and animals that excitement without physical exercise is capable of increasing the red count.

We have then all types of stimuli which may vary the number of red cells. It is apparent that the red count is subject to many great and rapid changes, that certain of these stimuli, such as exer-

³ As this paper was about to go to press, I told Professor Cannon of these results, and he kindly gave me a reference to the work of Ferrari, G. C.: *Ricerche globimetriche negli stati emozionali*, *Rivista di patologia nervosa*, 1897, ii, p. 306, who found in students taking examinations that the red count was above normal.

cise, asphyxia, fright, etc., are constantly occurring, and it appears certain that the number of red cells per unit volume of blood is not a fixed quantity, in the normal individual, but one which is constantly oscillating, depending upon his habits of life.

III. METHODS

(1) *The choice of a stimulus (epinephrin)*

It is of course conceivable that with different stimuli the changes in the red count may take place in different ways. An investigation of all the above mentioned stimuli would be too great a task for this paper. Epinephrin has therefore been chosen for nearly all experiments. The reason for doing this was that it is essential to find a stimulus which may be accurately graduated in amount. As epinephrin may be obtained in a pure state, the amount may be accurately graduated, and by intravenous injection the application of the stimulus carried out with certainty.

This is important where negative results are to be considered. In the use of such stimuli as lung emboli, asphyxia, improper application of these stimuli will fail to give positive results. They would then be improper stimuli to use in the following experiments, except as confirmatory of positive results.

Furthermore epinephrin occurs normally in the body, and as has been shown by many investigators is secreted from the adrenal glands in increased amounts under various physiological conditions.

Besides this, the action of epinephrin on the red count is very prompt, occurring in fifteen to twenty minutes, and is of sufficient magnitude to be recognized with great ease.

The object of all these experiments was more qualitative than quantitative, the idea being to obtain a reaction sensitive enough to be easily and promptly recognized. On this account careful and prolonged quantitative estimations of the number of red cells after various doses of epinephrin had been injected, and of the duration of such an increase, and more exact haemoglobin determinations than by the Sahli method, were not thought

necessary. Sufficient accuracy and care were used in these experiments to be well within the necessary limits of accuracy.

(2) *Technic of counting the red cells*

To obtain uniform counts, blood was drawn from a large vein, either the jugular or the femoral, by means of a small hypodermic needle, immediately forced out on a watch glass, and taken up in a Thoma-Zeiss pipette. The Thoma-Zeiss counting chamber was used.

Three pipettes which had been found to check on the same oxalated blood, and in a great many control experiments, were used and interchanged as regards order. The same slide and cover glass was always used, so that no relative error due to apparatus was present.

Two hundred small squares were always counted, in two drops, and if necessary a third drop was counted, and the three counts averaged. Having had a long hospital experience in counting red corpuscles, and having counted some five hundred samples of blood in these experiments, the results are I think fairly uniform. On account of the marked changes looked for no great stress was placed on changes under 500,000 although from calculation of the probable error in oxalated blood, and control animals under ether, the error was found to be much less than this.⁴

The experiments of Cohnstein and Zuntz (54), and others, have shown that the number of red corpuscles in the large arteries and veins is the same. Variation in the counts may however be rapidly brought about by obstruction to the blood flow. In these experiments I have either forced the needle with which the blood was obtained directly through the skin into the jugular vein, or in cases where the animal was to be sacrificed have exposed a vein and slipped the needle directly into it. By this method the blood flow was obstructed for a second or so only, and as I have found the same increase in the red count in experi-

⁴ For a complete discussion of methods of counting red cells, apparatus, and haemoglobin determinations, with the sources of error, see Bürker (53).

ments where the blood was taken from the ear, the great veins, the femoral artery, and from the heart itself, I feel certain that the results here given are a true index of the number of red cells per unit volume of blood in the general circulation.

(3) *The effect of ether on the red count*

Control experiments show that ether properly given does not raise the count. On the other hand if the animal has been excited during the giving of the anaesthetic, the count will be found high, and will gradually fall as the anaesthesia is continued. The effect then of ether excludes error of mistaking an increase in the number of red cells due to the anaesthetic.

Ether control count.

Experiment 16, January 9, 1915. Dog.

9.45. Reds, 7,592,000.

Ether.

11.00. Reds, 7,420,000.

11.30. Reds, 7,644,000.

12.46. Reds, 7,832,000.

1.22. Reds, 7,592,000.

Asphyxia for twenty minutes.

1.27. Reds, 8,408,000.

(4) *The effect of epinephrin on the red count*

If a dog or cat is etherized, a count taken from the jugular vein, epinephrin in doses of 0.9 mgm. per kilo given intravenously in some other vein, and counts again taken at fifteen minute intervals, it will be found with great regularity that the red cells will increase from one million to two millions per cubic millimeter of blood in from fifteen minutes to thirty minutes, and then gradually fall. At times the count does not reach its maximum for thirty minutes, but if the count rises before this, it is maintained at its height for at least thirty minutes after the injection. This dose, chosen at random, has been found to give very uniform results. No attempt has been made to quantitate the

effect of injections of epinephrin in various doses, as it was unnecessary in this type of experiment.⁵

The results of epinephrin injections in a normal dog are shown below.

Experiment 17, June 1, 1915. Dog, 11.3 kilo.

Reds, 8,000,000.

Epinephrin 0.9 mg. per kilo, intravenously.

Reds, 10,184,000, 19 minutes after injection.

Experiment 18, June 3, 1915. Dog, 13.4 kilo.

Reds, 8,000,000.

Epinephrin 0.9 mg. per kilo, intravenously.

Reds, 9,840,000, 23 minutes after injection.

IV. THE PART PLAYED BY THE DIFFERENT ORGANS IN ACUTE POLYCYTHAEMIA

Let us consider the possibility of any one organ being able to act either as a reservoir for red cells, a factory for producing new red corpuscles, or an apparatus by means of which the blood may be sufficiently concentrated to give the required relative increase in the number of red cells.

The spleen and the bone marrow are generally considered as the seat of red cell formation in the adult. The splanchnic area has been much spoken of as a stagnant area in which corpuscles may sediment (55).

The kidneys, the great capillary areas of the lungs, the muscles, mesentery, liver, spleen, intestine, and the skin, may be considered as surfaces from which fluid may very rapidly be taken from the circulation and either excreted or stored temporarily in the lymphatic system.

Experiments have been undertaken systematically by me to find out if possible the part played by these various organs in the production of polycythaemia.

⁵ The epinephrin used in these experiments was prepared by Professor Abel, according to the method described by him in the *Berichte der Deutsch. Chem. Gesellschaft*, Vol. 36, p. 1841, 1903 and contained only inorganic matter as an impurity.

(1) *The part played by the intestine, its mesentery, and the omentum*

If an animal is etherized, the entire intestine with its mesentery, and the omentum removed, it will be found that subsequent injection of epinephrin in doses of 0.9 mg. per kilo will produce an increase in the number of red cells as in a normal animal.

Experiment 19, May 7, 1915. Cat, 3.0 kilo.

10.45. Ether.

11.20. Operation finished. Entire intestine, its mesentery, and the omentum removed.

11.30. Reds, 9,584,000.

11.32. Epinephrin 0.9 mg. per kilo, intravenously.

11.45. Reds, 10,880,000.

12.10. Reds, 10,456,000.

Experiment 20, May 7, 1915. Cat, 3.8 kilo.

2.15. Ether.

2.20. Reds, 11,400,000.

2.42. Operation finished, entire intestine, its mesentery, and the omentum removed.

2.45. Reds, 11,640,000.

2.47. Epinephrin 0.9 mg. per kilo, intravenously.

3.05. Reds, 13,320,000.

3.27. Reds, 14,024,000.

Experiment 21, May 24, 1915. Dog, 9.0 kilo.

2.05. Ether.

2.23. Operation finished, entire intestine, its mesentery, and the omentum removed.

2.28. Reds, 9,296,000.

2.29. Epinephrin 0.9 mg. per kilo, intravenously.

2.50. Reds, 9,760,000.

3.07. Reds, 10,216,000.

3.30. Reds, 11,960,000.

It is apparent then that removal of the intestine, its mesentery, and the omentum have no appreciable effect on the action of epinephrin in raising the red count.

(2) The part played by the spleen

Similarly if the abdomen is opened and the spleen removed it will be found that injection of epinephrin subsequent to removal of the spleen is followed by an increase in the number of red cells as in a normal animal.

Experiment 22, July 14, 1915. Dog, 8.3 kilo.

2.55. Ether.

3.05. Operation finished, spleen removed.

3.10. Reds, 8,024,000.

3.11. Epinephrin 0.9 mg. per kilo, intravenously.

3.32. Reds, 10,040,000.

(3) The part played by the pancreas

And finally if the intestine, its mesentery, the omentum, spleen and pancreas, are all removed at one operation, it will be found that this has no influence on the ability of epinephrin to produce an increase in the number of red cells subsequent to the removal of these organs.

Experiment 23, May 31, 1915. Dog, 6.5 kilo.

2.30. Ether.

3.20. Operation finished. Entire intestine, its mesentery, the omentum, spleen and pancreas removed.

3.23. Reds, 6,196,000.

3.24. Epinephrin 0.9 mg. per kilo, intravenously.

3.40. Reds, 7,360,000.

3.57. Reds, 7,544,000.

4.20. Reds, 7,328,000.

(4) The part played by the liver

On account of the complicated double blood supply to the liver it is impossible to remove it and to leave the rest of the circulation intact, without resorting to mechanical contrivances. Some of these were tried but given up on account of the introduction of too many complications.

It is however possible to judge of the part played by the liver from various experiments.

(a) *Exclusion of the liver from the circulation.* In the first place if the chest is opened by an incision between the eighth and ninth ribs, the skin and all tissues cut to the sternum, and to the backbone, secure ties placed about the aorta, and vena cava, just above the diaphragm, and about the sternum, and back bone, including the azigos veins, we have an animal which is cut in two above the diaphragm, except for what blood can pass up and down through the vertebrae and their contents.

In such an animal, injection of epinephrin in the ordinary dose (0.9 mg. per kilo intravenously) causes no increase in the number of red cells.

Experiment 24, May 11, 1915. Dog.

11.15. Ether.

12.32. Operation finished. Animal tied in two above the diaphragm.

12.33. Reds, 7,024,000.

12.34. Epinephrin 0.9 mg. per kilo, intravenously.

12.49. Reds, 6,504,000.

12.58. Reds, 6,600,000.

Experiment 25, May 17, 1915. Dog.

11.58. Ether.

12.20. Operation finished. Animal tied in two above the diaphragm.

12.29. Reds, 6,880,000.

12.31. Epinephrin 0.9 mg. per kilo, intravenously.

12.50. Reds, 6,200,000.

1.05. Reds, 5,504,000.

1.20. Reds, 5,784,000.

Experiment 26, May 18, 1915. Cat.

10.10. Ether.

10.45. Operation finished. Animal tied in two above the diaphragm.

10.50. Reds, 10,616,000.

10.55. Epinephrin 0.9 mg. per kilo, intravenously.

11.16. Reds, 9,976,000.

11.35. Reds, 9,496,000. Cat in excellent condition.

Experiment 27, May 18, 1915. Cat.

12.00. Ether.

12.40. Operation finished. Animal tied in two above the diaphragm.

12.45. Reds, 9,136,000.

12.50. Epinephrin 0.9 mg. per kilo, intravenously.

1.13. Reds, 8,816,000.

1.40. Reds, 8,680,000.

In these experiments there was practically no loss of blood from the operation, and the animal lived for hours in excellent condition.

It is necessary to tie *everything*, not only the sternum and great vessels, but the skin must be divided completely so that there is no anastomosis between the upper and lower half of the body, otherwise an increase in the count may take place.

(b) *The effect of bringing the liver into the circulation.* If we open the abdomen by lateral incisions and cut the skin to the back bone, place a tie about the aorta just below the mesenteric artery, and above the renals, and a tie about the inferior vena cava just below the liver, and finally pass a tie about the aorta, and about the veins and tissues adjacent to the back bone, and tie the whole securely, we have an animal which is again tied in two, but in this case the blood supply has been left intact to the intestines, stomach, spleen and liver. *As removal of all of these organs except the liver has no effect on the production of epinephrin polycythaemia, we have in this case merely added the liver to the animal which we divided above the diaphragm.*

In such an animal the injection of epinephrin causes the customary polycythaemia.

Experiment 28, May 18, 1915. Cat.

2.20. Ether.

4.00. Operation finished. Abdomen opened by lateral incisions to back bone. Tie placed about the aorta just above the renals, and below the mesenteric. Also about the vena cava just below the liver. Secure tie then placed about the aorta at the same level as the first tie, and about the backbone with the adjoining tissues.

4.12. Reds, 9,864,000.

- 4.13. Epinephrin 0.9 mg. per kilo, intravenously.
- 4.32. Reds, 10,520,000.
- 4.48. Reds, 11,760,000.

(c) *The effect of epinephrin after ligation of the arterial blood supply to the liver.* Experiments were then undertaken to interrupt the arterial blood supply to the liver by operations on the hepatic artery.

The first of these was done while attempting to remove all of the abdominal organs including the liver. The hepatic artery was accidentally cut. The coeliac axis artery was clamped, thus shutting off all arterial blood supply to the liver. (Except for possible abnormal anastomosis.) *In this case injection of epinephrin caused no increase in the number of red cells as will be seen from the following experiment.*

Experiment 29, May 25, 1915. Dog.

- 9.35. Ether.
- 10.32. Operation finished. Stomach, gut, mesentery, spleen and pancreas removed. *Coeliac axis artery clamped in abdomen.*
- 10.34. Reds, 7,944,000.
- 10.35. Epinephrin 0.9 mg. per kilo, intravenously.
- 11.00. Reds, 7,360,000.
- 11.14. Reds, 7,576,000.
- 11.36. Reds, 7,736,000.

On account of the hepatic artery having an irregular number of branches, it was considered dangerous to attempt to tie this artery. As we have seen that removal of the organs supplied by the coeliac axis artery, with the exception of the liver, has no effect on the production of polycythaemia on subsequent injection of epinephrin, we may shut off the arterial blood supply to the liver by tying this artery, and consider the result of this operation as affecting the liver alone, as far as an increase in the number of red cells is concerned.

To avoid all interference with the abdominal organs, and to obtain a clean exposure of the coeliac axis artery, the chest was opened on the left between two of the lower ribs, under artificial respiration. The coeliac axis artery branches from the aorta

between the pillars of the diaphragm and may be reached with great ease and practically no dissection by this incision. Having thus exposed the artery, the following experiments were done.

Experiment 30, May 26, 1915. Dog, 4.5 kilo.

10.25. Ether.

11.00. Operation finished. Coeliac axis artery tied.

11.02. Reds, 5,656,000.

11.03. Epinephrin 0.9 mg. per kilo, intravenously.

11.23. Reds, 5,704,000.

11.35. Reds, 6,655,000.

1.05. Reds, 5,176,000.

Experiment 31, May 26, 1915. Dog, 6.6 kilo.

1.45. Ether.

2.20. Operation finished. Coeliac axis artery tied.

2.21. Reds, 7,096,000.

2.24. Epinephrin 0.9 mg. per kilo, intravenously.

2.48. Reds, 7,144,000.

3.00. Reds, 7,040,000.

3.25. Reds, 6,680,000.

Thus ligature of the coeliac axis artery prevents polycythaemia after the injection of epinephrin.

(d) *The effect of removing this ligature some time after the injection of epinephrin.* It was then thought that if the coeliac axis artery were clamped, epinephrin injected, and no polycythaemia were produced, removal of this clamp would then restore the arterial circulation to the liver, and might possibly affect the number of red cells. To investigate this point the following experiments were done.

Experiment 32, May 27, 1915. Dog, 5.9 kilo.

10.00. Ether.

10.17. Clamped coeliac axis artery.

10.29. Reds, 7,544,000.

10.30. Epinephrin 0.9 mg. per kilo, intravenously.

10.45. Reds, 7,416,000.

11.01. Reds, 7,360,000.

11.03. *Clamp removed.*

11.18. Reds, 8,640,000.

11.31. Reds, 9,280,000.

Experiment 33, May 29, 1915. Dog, 5.9 kilo.

10.45. Ether.

11.30. Coeliac axis artery tied with tape.

12.17. Reds, 8,696,000.

12.18. Epinephrin 0.9 mg. per kilo, intravenously.

12.34. Reds, 8,904,000.

12.50. Reds, 9,040,000.

12.55. *Removed tie from artery.*

1.14. Reds, 10,936,000.

1.27. Reds, 10,424,000.⁶

It appears then that shutting off the arterial blood supply to the liver through the hepatic artery prevents an increase in the number of red corpuscles after the injection of epinephrin; that when this circulation is again established even one-half hour after the injection of epinephrin, the red cells increase in number exactly as if the usual dose of epinephrin had been injected into a normal animal.

As a control experiment, to be certain that interference with the arterial blood supply to the liver caused no marked change in the red count, an experiment was done under exactly the same conditions as above, that is, the chest was opened, a ligature tied about the coeliac axis artery, and counts done. One-half hour later the tie was removed and further counts done. Removal of the tie had no great effect on the blood count.

Experiment 34, May 31, 1915. Dog, 7.5 kilo.

10.30. Ether.

11.20. Operation finished, coeliac axis artery tied.

11.25. Reds, 8,840,000.

11.42. Reds, 8,080,000.

11.58. Reds, 8,416,000.

Tie removed.

⁶ In two experiments in which a *clamp* was used, there was a temporary rise in the number of red cells after injection of epinephrin, but this fell 15 minutes later, and was followed by a rise of 1,168,000 and 1,338,000 respectively, as soon as the clamp was removed. This temporary rise is very different from anything which occurred previously, as any rise remains high for at least 30 minutes. It may probably be accounted for by slipping of the clamp or a slight leak during the very great initial pressure on epinephrin injection, as there was in no instance any such rise when the artery was ligatured.

12.25. Reds, 8,576,000.

12.40. Reds, 9,024,000.

1.05. Reds, 8,640,000.

It is apparent then that the arterial blood supply to the liver is necessary for the production of polycythaemia after the injection of epinephrin. Although blood with large amounts of epinephrin must reach the liver cells by way of the portal vein, there is no change in the red count. But as soon as the blood is allowed to flow again, through the hepatic artery, the count immediately rises.

(5) *The part played by the bone marrow, muscles, lungs, kidneys, and skin*

In the experiments in which the coeliac axis artery was tied, and in which the animal was tied in two above the diaphragm, where there was no polycythaemia after epinephrin injection the bone marrow, lungs, muscles, and skin were unable to increase the number of red cells.

The same may be said of the kidneys in those experiments in which the coeliac axis artery was tied and in which the blood supply to the kidneys was in no way interfered with. Besides this it has been repeatedly noticed that there has been no increase in the amount of urine in the bladder during the production of polycythaemia.

That the increase in number of red cells in dogs and cats can be due to loss of fluid by the skin is of course excluded by the absence of sweat glands, except in the feet.

We see then that it is the liver which is responsible for the increase in the number of red cells brought about by the injection of epinephrin, and that the other tissues are incapable by themselves of suddenly varying the red count.

V. CHANGES OCCURRING IN THE LIVER BY WHICH THE NUMBER OF RED CELLS IS INCREASED

As I have shown before, the increase in the number of red cells must take place by one of the following methods: a production

or splitting of the red cells, loss of fluid from the blood, a pouring out of corpuscles packed away in some organ, or a combination of these methods.

(1) *Changes in the plasma or blood volume*

Let us first take up changes in volume of the blood or plasma, and see if these changes alone will account for the variation in number of red cells which has been found.

Concentration of the blood, or a decrease in blood volume, has been studied by the following methods.

- (a) Determination of the total solids.
- (b) Electrolytic, cryoscopic, and refractometric methods.
- (c) The carbon monoxide method.
- (d) Dye method.

(a) *Determination of the total solids.* The method here used has been to determine, during some experimental procedure, the change in weight of a unit volume of dried blood. An increase in the total solids was looked upon as a decrease in blood volume, and a decrease in the total solids as an increase in blood volume. As the corpuscles are of higher specific gravity than the plasma, it will readily be seen that a relative increase in the number of corpuscles, with no change in the total blood volume, will increase the total solids of the blood. This method alone is then no index of a change in blood volume.

(b) *Electrolytic, cryoscopic, and refractometric methods.* Determination of the solids in the plasma, by changes in the freezing point, electrical conductivity, or refractometric measurements, will indicate exactly any variation in the concentration of electrolytes, or in the molecular concentration, but will not of course account for this change. An increased secretion of solid matter into the circulation, as for example sugar, will increase certain of these factors, but no change in the blood volume will of necessity have taken place.

We are forced then to give up these methods as an index of changes in the blood volume.

(c) *Carbon monoxide method.* This method consists in breathing a known volume of carbon monoxide gas, and determining its dilution in the blood (56). As the carbon monoxide is diluted in the haemoglobin, which in turn is almost entirely in the red corpuscles, this method is dependent upon the ability of the carbon monoxide to obtain an even distribution in these corpuscles. If, as has been supposed, and as is physiologically possible, corpuscles are packed away in certain areas, it is conceivable that the carbon monoxide will not reach all of these corpuscles. Whether this does actually occur or not is just the point under discussion, and on this account for study of acute changes in blood volume during the production of polycythaemia other methods were used.

(d) *Dye method.* One method still remains and that is the determination of some substance which may be carried by the plasma. The ideal substance would be one soluble in the plasma, non-toxic, and incapable of absorption or excretion by the tissues or corpuscles. Such a substance has not yet been found. The nearest approach to this is the method worked out by Rowntree, Keith and Geraghty (57) in which a substance of colloidal nature is injected into the blood stream, and its dilution in the plasma estimated by colorimetric methods. Haematocrit determinations give the relative volumes of corpuscles and plasma, and from this the total blood volume may be estimated. This method is open to the theoretical criticism that a variable amount of dye may be picked up by the tissues, destroyed or excreted. Practically, the method appears to give very constant results, and as the dilution of the dye takes place in the plasma, and not in the corpuscles, it is a method of particular importance in this type of experiment.

Dr. Keith kindly estimated the plasma volume and total blood volume for me in two dogs, before and after the injection of epinephrin. The results of these experiments are given below.

Experiments 35 and 36.

The dogs weighed 11.5 and 13.4 kilo respectively. Were etherized, counts were done, and blood drawn for analysis from the jugular vein.

Epinephrin in doses of 0.9 mg. per kilo was given intravenously. 39 and 30 minutes respectively after the injection, blood was again drawn from the other jugular vein for counts and analysis. The results are tabulated below.

REDS IN MILLIONS		REL. OF REDS TO PLASMA		PLASMA VOLUME		TOTAL BLOOD VOLUME		AV. VOL. OF RED CELLS	
Before	After	Before	After	Before	After	Before	After	Before	After
		<i>per cent</i>	<i>per cent</i>						
8.0.....	10.2	48.5	53.5	623	540	1209	1161	606	524
		51.5	46.5						
		44.3	47.7	755	609	1355	1164	606	522
8.0.....	9.84	55.7	52.3						

Percentage changes in the above experiments

REDS	PLASMA VOLUME	TOTAL VOLUME	AV. RED CELL VOLUME	HAEMOGLOBIN
<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>	<i>per cent</i>
28+	13.3—	3.97—	13.8—	
23+	19.3—	14.0—	12.9—	13+

In these two experiments it will be seen that there is a distinct decrease in the plasma volume, almost sufficient in one case to account for the increase in the number of red cells, but in the other experiment there is a wide margin between the percentage increase in red cells and decrease in plasma volume. Although more experiments would be necessary to fix with certainty the exact relation between the change in the number of red cells, haemoglobin and the blood volume, these experiments and evidence found in the literature show with a fair degree of certainty that changes in the plasma volume cannot entirely account for the changes observed in the number of red cells.

Experiments done by Abderhalden, Dreyer and Walker, Douglas, Haldane, Henderson and Schneider (58), at high altitudes by the carbon monoxide method give very varying results, as regards changes in blood volume. In certain instances,

a decrease in the blood volume is found, in others no change, and in still others an increase in the blood volume. On account of the high degree of accuracy with which these experiments were carried out, the variable results might possibly be attributed to the changes in the distribution of the corpuscles in the body affecting the volume determinations as I have suggested above (p. 197). Or, as the experiments carried out by these observers were not of the acute type (that is, of a few minutes duration), the variation in results may be due to secondary changes. Finally as has been observed by most previous investigators different individuals react differently. Reasons for the individual variations are brought out in the following pages. One point stands out clearly however in all of these experiments, and that is that the changes in blood volume cannot entirely account for the increase in the number of red corpuscles.

This being the case we must have, to a certain degree at least, a production of new red cells, a splitting of these, or areas in the body where the cells are packed away in greater concentration than in the circulating blood.

(2) *Changes in the red corpuscles themselves*

In the first place let us see if there is any indication of change in the corpuscles themselves during polycythaemia.

(a) *The question of red cell division.* That the percentage increase in haemoglobin is not equal to that of the red cells is a fact well known to nearly all observers (59). This fact is important as it shows that the increase in red cells is not entirely due to a decrease in plasma volume. Some changes must take place in the red cells, or other red cells with different properties must enter the circulation.

Besides this, one constantly runs across the statement that microcytes have been seen in smears made at high altitudes. Having the data present in experiments 35 and 36 to determine the volume of the individual corpuscles before and during polycy-

thaemia this calculation was made and gave the following results.

The average volume of the individual red cell before polycythaemia in each of the two experiments was 606^{-8} and during polycythaemia 524^{-8} and 522^{-8} respectively, showing that the average volume of the red cells diminished 13.4 per cent during polycythaemia.

This would indicate that unless all the corpuscles were diminished in size the increase in number of red cells was due in part to red cells distinctly smaller than normal.

If we return once more to the haemoglobin content of the corpuscles, it will be seen that if an increase in the number of red cells actually does take place, when there is no increase in the haemoglobin, there must be a division of red cells or red corpuscles containing no haemoglobin must be brought into the circulation. From certain data obtained from my own experiments and from those found in the literature, it seems that such a condition does take place. Further experiments using exact haemoglobin methods are being undertaken before concluding definitely that division of the red cells takes place in polycythaemia.

There is however proof that cells are present during polycythaemia which were not there before its production. The question then arises as to whether these are new red cells, or as to whether they are cells brought out into the circulation from reservoirs in the body.

(b) *The question of red cell formation in acute polycythaemia.* There are various methods which have shown at times of blood regeneration certain cells in the circulating blood, not normally present, which on account of their staining properties or their functional behavior are different from the normal. From collected evidence it has been assumed that these cells indicate a new type, or young red cells.

The means of studying these cells may be divided into functional and histological methods.

(1) *Functional methods.* (a) *Changes in the fragility of the red corpuscles.* Changes in the fragility of the red cells have been looked upon as an indication of new red cell formation. On this account the following experiment was done to find out if there were any change in the fragility of the red cells during polycythaemia. As will be seen from an examination of the figures, no change in the fragility of the red corpuscles was observed during an acute polycythaemia produced by the injection of epinephrin.

Experiment 37, July 8, 1915. Dog, 3.7 kilo.

Salt solutions were made up in strengths of 0.75 per cent to 0.25 per cent with differences of 0.05 per cent in each. The dog was etherized and blood drawn from the jugular vein into oxalate, centrifuged, washed and suspended in 0.80 per cent solution. At the same time a count was taken. Epinephrin was then injected, 0.9 mg. per kilo. Thirty-four minutes later blood was again drawn and treated as before.

The counts before and after injection were 6,136,000 and 7,296,000 respectively. After forty-five minutes' incubation at 37°C. haemolysis was present in the solutions of 0.25–0.35 per cent and a trace was noticed in the 0.40 per cent solution in *both* bloods.

On standing overnight at room temperature, complete haemolysis was seen in the 0.25–0.40 per cent and slight haemolysis in the 0.45 per cent tubes. None in the other tubes. *Both* bloods showed the same degree of haemolysis.

(b) *Metabolism of the corpuscles themselves.* It has been shown that besides substances capable of oxidation, normally found in small amount in the blood (60), the red corpuscles have, quite independently of these substances, an ability to use up a certain amount of oxygen in their own metabolism (61); furthermore in blood after haemorrhage, containing nucleated red cells, and in animals in which the red cells are normally nucleated, as in the goose, that this metabolism of the corpuscles themselves is increased (62). This fact has been used to determine the presence of young cells in the blood after haemorrhage, and in conditions where the presence of young cells is desired to be determined. Morawitz and Masing made determinations of this kind at Col d'Olen (10,000 feet) and were unable to show any increase in the metabolism of the blood at this altitude, from which they conclu-

ded that the increase in red cells occurring at this altitude was not due to young red corpuscles (63).

I have carried out similar experiments on the blood of dogs, before and during the production of polycythaemia by the injection of epinephrin. The method used was as follows.

Blood was drawn from the jugular vein, defibrinated and saturated by shaking with oxygen. Three samples of this blood were then placed in small sterilized glass tubes containing a glass bead for shaking. Stoppered and placed in the incubator at 37° .

Epinephrin in doses of 0.9 mg. per kilo was injected into the saphenous vein. Counts were made before and fifteen or twenty minutes after the injection of epinephrin. Blood was again drawn from the jugular vein during the polycythaemia, and treated exactly as that taken before the injection of epinephrin.

The blood was then analyzed in all of these samples, before and after the injection of epinephrin, and before and after incubation.

The apparatus used was Barcroft's micro differential gas manometer (63)a.

0.2 ccm. of a 0.4 per cent ammonia solution was placed in each bottle. 0.1 ccm. of blood was run under the ammonia from a fine 0.2 ccm. pipette. The blood was then haemolyzed, a drop of ferricyanide added to the receiver, and the apparatus placed in a water bath kept with great accuracy at 37.8° .

After five minutes the cyanide was mixed with the blood, and readings taken with several shakings until a constant reading was obtained. The bottle was then removed, a drop of cyanide added to the receiver on the opposite side, and a second analysis done in the same way.

After a definite time the blood was removed from the thermostat. Shaken well. 0.3 ammonia was placed in one bottle and 0.2 ccm. in the other. Blood was then quickly drawn up in the pipette, from the bottom of the tube, which allowed contact with the air by the upper part of the column of blood only. As the pipette had a 3 cm. capillary end, practically no air could reach the blood from below in the short time taken for the measurement. A little blood was allowed to run out of the pipette, and then 0.1 ccm. was run under the 0.2 ccm. of ammonia, leaving a long column of blood behind in the pipette. In this way there was little chance for the blood to become resaturated.

As all of these experiments were done in the same way, the results are relatively accurate. No calibration or transferring to amounts of oxygen was necessary, but the results are expressed in centimeter pressures of oxygen, and in percentages of oxygen used before and during polycythaemia.

Experiment 38, July 2, 1915. Dog, 8.5 kilo.

8.30. Ether.

8.50. Reds, 8,576,000.

9.04. Blood drawn for analysis.

9.09. Epinephrin 0.9 mg. per kilo, intravenously.

9.26. Reds, 9,864,000.

9.46. Reds, 9,744,000.

9.45. Blood for analysis.

Before Epinephrin

3.81 cm. oxygen pressure.

3.01 cm. oxygen pressure after

5.5 hours' incubation.

Reduction 26.5 per cent.

After Epinephrin

3.90 cm. oxygen pressure.

3.04 cm. oxygen pressure after

5.5 hours' incubation.

Reduction 26.3 per cent.

Experiment 39, July 3, 1915. Dog, 6.6 kilo.

8.15. Ether.

9.29. Reds, 7,704,000.

9.33. Blood drawn for analysis.

10.01. Epinephrin 0.9 mg. per kilo, intravenously.

10.14. Reds, 9,224,000.

10.34. Reds, 9,760,000.

10.37. Blood drawn for analysis

Before Epinephrin

3.54 cm. oxygen pressure

2.75 cm. oxygen pressure after

5.0 hours' incubation

Reduction 22 per cent.

After Epinephrin

3.91 cm. oxygen pressure.

3.23 cm. oxygen pressure after

5.0 hours' incubation.

Reduction 17.4 per cent.

From these experiments it is seen that the oxygen content of the blood is increased during polycythaemia as might be expected on account of the increase in haemoglobin, but that there is no increased metabolism of the red cells present, during epinephrin polycythaemia. In fact in one experiment there was less oxygen used by the red cells present in polycythaemia than was used by the red cells before its production.

(2) *Histological methods.* In polycythaemia at high altitudes, nucleated red cells have been observed by a few authors (64). Nearly all authors are however unanimous in the belief that there is no increase in these cells under such conditions. In my own experiments repeated studies of smears made with Wright's stain have shown no nucleated red cells. Besides this, stains have been made with cresyl blue (65). According to Musser and Krumbhaar (66) reticulated cells staining by this method are present in the dog normally from 1.0 to 4 per cent. I attempted to determine the number of these cells present before and during the production of polycythaemia by the injection of epinephrin, thinking that on account of the small number normally present an increase could be easily recognized. In one dog during an increase of 1,400,000 in the red count there was an increase in the number of these cells from one in every two fields to two in each field. A control dog merely placed under ether showed as many as 14 of these cells in each field, before any epinephrin was given. It is, however, worth mentioning that the count in this dog was 9,248,000.

It was immediately evident that to determine accurately the number of these cells present would need careful counting methods. As these experiments were undertaken only shortly before this paper went to press there was not time to do this. It was merely thought proper to mention that in both these dogs, one after epinephrin injection, and the other with a high red count, the number of these reticulated corpuscles appeared to be much above the normal.

(3) *The question of a reservoir of red cells*

As regards the question of a reservoir, from which red cells may emerge at times, we have seen that it is one of the logically possible ways in which the number of red corpuscles per unit volume of blood might be increased. Whether such a reservoir actually exists or not, and how great a rôle this plays in polycythaemia, is still open to question.

Most investigators have considered the possibility of sedimen-

tation of red cells in the capillaries. Cohnstein and Zuntz (67) observed the flow of red cells in the frog's mesentery, and counted the corpuscles in the large vessels and in the capillary areas of the extremities, in various circulatory conditions. They concluded that the red count in the capillaries varied with changes in the circulation through them. On this account massage was thought of as a means of forcing on the corpuscles lying in the stagnant areas of the limbs and the mesentery. Massage of the limbs and of the abdomen has been found by many investigators to cause a considerable increase in the number of red cells (68). In the experiments where abdominal massage was carried out, the "splanchnic area" was spoken of as the place of stagnation of red cells, but there is no mention made of any more exact location than this.

As massage of the abdomen is to all practical purposes direct massage of the intestines and mesentery, it occurred to me that the question of these organs being the seat of the theoretical reservoir could be easily determined by removal of them. As is shown in experiments 19, 20, 21, and 23, removal of the intestine and its mesentery has no effect on the production of polycythaemia after epinephrin injection. That these organs acting as a reservoir is the chief source of red cells in polycythaemia is therefore excluded.

Massage of the abdomen must then cause a polycythaemia by other means. We have seen that the liver is the source of the red cells in epinephrin polycythaemia, and that the increase in number of red cells is dependent upon the blood flow through the hepatic artery. It would then appear unlikely that an increased flow of blood through the portal vein, as occurs from massage of the intestine in abdominal massage, would greatly affect the red count.

The possibility of abdominal massage physically forcing red cells from the liver, as it might from the intestine and mesentery, is of course highly improbable, on account of the liver's protected position under the ribs. However to be certain of this point the abdomen was opened by a median incision and direct massage of the liver carried out by manipulation of the various lobes between the fingers, taking care to disturb the other organs as lit-

tle as possible. It will be seen from experiment 40 that massage did not physically force the red cells from the liver, as the red count was lower after twenty minutes of massage than before.

The idea then that massage increases the red count by physically forcing the red cells from a reservoir in the abdomen must be abandoned, and its effect on the number of red corpuscles sought elsewhere. The complicated reflex mechanism by which the number of red cells may be increased will be taken up later.

Experiment 40, July 9, 1915. Dog, 7.5 kilo.

8.23. Ether.

8.34. Reds, 8,144,000.

8.35-8.55. Massage of the liver by manipulation of the various lobes with the fingers, through an incision in the abdomen.

9.00. Reds, 7,176,000.

9.21. Reds, 8,424,000.

9.37. Reds, 7,520,000.

10.05. Reds, 7,221,000.

(4) *Summary of the part played by the liver*

If then we sum up the evidence as to the method by which the liver is capable of increasing the number of red cells (knowing that it is the liver alone in epinephrin polycythaemia which causes the number of red cells to increase, and seeing, as we shall, that in the other conditions spoken of we cannot rule out an increase in the epinephrin output) we find:

(1) That the plasma volume is decreased, but not enough to entirely account for the increase in the number of red cells.

(2) That cells are present in acute polycythaemia which are not present before its production. This is shown by the decrease in average volume of the red cells and their reduced haemoglobin content.

(3) There is no evidence of red cell formation by

(a) The presence of nucleated red cells.⁷

(b) Changes in fragility of the corpuscles.

(c) Increased metabolism of the corpuscles.

⁷ Nucleated cells have been found by very few authors, see page 204.

(4) Rare experiments indicate a division of the red cells on account of an increase in the number of red cells with no changes in the haemoglobin content of the blood.

As to how the liver brings about these changes in the number, volume, and size of the corpuscles, or what ligature of the hepatic artery does to interfere with this change, I have as yet made no attempt to ascertain.

VI. THE MECHANISM CONTROLLING THE RED CORPUSCLE CONTENT OF THE BLOOD

(1) *Reasons for assuming such a mechanism to be present*

We are so accustomed to thinking that a normal animal has under normal conditions a practically constant number of red cells per unit volume of blood that we are accustomed to very generally use the terms, normal count, an anaemia, and a polycythaemia, indicating changes from the normal.

When any variable factor is maintained constant under varying conditions, one is justified logically in assuming the presence of a regulatory mechanism for the maintenance of this constancy.

On a superficial study of the red counts, one would be led to look for some regulatory mechanism the purpose of which is to maintain a constant number of red corpuscles per unit volume of blood. On more careful study it is found that this is just what the body does not do. The red count is constantly varying under normal conditions, and it is maintained at a different level for long periods of time when occasion arises. It is not then the number of red cells or the amount of haemoglobin which is maintained constant. But it is evident that it is some *function* of the red cells which is the factor regulated. The chief function of the red cells known to us at present is their ability to carry oxygen by means of their haemoglobin, the oxygen capacity of the blood varying nearly exactly with its haemoglobin content.

We know however that in the body the amount of oxygen per unit volume of blood need not necessarily vary with its haemoglobin content, the reason for this being that on account of the

varying oxygen tensions in the inspired air, variation in the capillary surface of the lungs, in pathological conditions, and variation in the rapidity of blood flow through the lungs, the oxygenation of the blood is not always complete. In certain such conditions, it is evident that if the haemoglobin surface were increased, as by the increase in the number of corpuscles, together with an increase in the haemoglobin content of the blood, not only would the oxygen carrying capacity be increased, but also the chance for the blood to obtain more oxygen in the unit of time.

We see then that in the case of an acute polycythaemia, with the increase in the number of red cells, a diminution in their average volume, and an increase in the haemoglobin content of the blood, conditions are made more favorable for the blood to obtain more oxygen in cases where the consumption is increased, and to obtain the necessary amount where there is interference with the normal supply.

A sudden asphyxia will increase the red count temporarily. A continued asphyxia at high altitudes will maintain an increased number of red cells, and a return to the normal oxygen supply will be followed by a return to the normal count. It is apparent then that this mechanism which does control the oxygen carrying capacity of the blood reacts with great certainty to variations in the oxygen supply to the body.

How then does this decreased oxygen content of the blood act on the liver, to cause the increase in the red count?

This might occur in one of three ways—

- (1) By directly acting on the liver cells.
- (2) By a reflex nervous stimulation of the liver.
- (3) By the production of some substance in the body, which in turn could be carried to the liver, and act there directly.

(2) *The nervous control of the red count*

When this work was first undertaken, and before it was found that the liver was the seat of the changes which cause the increase in red cells, certain experiments were done to deter-

mine if possible whether the asphyxia produced by lung emboli acted generally throughout the body to cause the red cells to increase, or whether there was some center in the brain which, by means of nervous control, regulated the count. All experiments dependent upon nerve stimulation gave very varying results, and consequently another stimulus was sought which would be more certain in its action. As we have seen epinephrin was found, and proved to be exceedingly satisfactory for the purposes for which it was used. The question of a nervous center controlling the red count was neglected for the time being, but during the course of these experiments certain facts were discovered which pointed to a nervous control of the red count. Although these experiments have not been carried far enough to settle this complicated problem, they will I think throw some light on the subject.

If we now go over the results of the experiments carried out, with the purpose of discovering if possible the nervous mechanism controlling the red count, certain points will stand out very clearly.

(a) *Cerebral asphyxia*. In the first place, asphyxia of the head alone was attempted, to ascertain if there were a center there, capable of reacting to local asphyxia and reflexly causing an increase in the number of red cells. This was carried out by ligaturing the carotid arteries, and the jugular veins. In one animal (experiment 41) the result was very striking, a gradual rise of over four million taking place after ligaturing these vessels and *falling immediately* on removing the ties and restoring the circulation to the head.

A second such experiment was done which showed an increase in the red count after clamping vessels to the head, but in which the increase in number of red cells was very much smaller and was followed by no marked drop on removal of the clamps.

Control experiments were then undertaken. In the first of these the great vessels of the neck were exposed, but not tied. Counts were then done during the day. It was found that the increase in red cells was about as great as when the vessels to the head were tied (experiment 43).

These animals had been given chloretone intraperitoneally as an anaesthetic. A control of the effect of this on the red count was done, which showed that no marked change in the number of red cells took place under the influence of this anaesthetic over a period of three days (experiment 44).

(b) *The stimulation of certain nerves.* It appeared then that irritation of the neck was as effective a stimulus to the production of polycythaemia as asphyxia of the head.

Experiment 41, January 12, 1915. Dog, 5.3 kilo.

Chloretone 1.3 gm. dissolved in oil and given intraperitoneally.

11.55. Reds, 8,616,000.

12.08. Both carotid arteries and jugular veins ligatured.

12.28. Reds, 9,144,000.

1.03. Reds, 11,512,000.

1.30. Reds, 10,896,000.

2.40. Reds, 11,136,000.

4.20. Reds, 12,960,000.

All ligatures taken off.

4.50. Reds, 9,520,000.

5.15. Reds, 9,608,000.

Experiment 42, January 13, 1915. Dog. 8.5 kilo.

11.09. Chloretone. Intraperitoneally. 3.0 gm.

11.50. Reds, 6,328,000.

11.55. Both carotid arteries and jugular veins tied.

12.50. Reds, 7,248,000.

1.50. Reds, 7,888,000.

3.30. Reds, 7,312,000.

5.37. Reds, 7,688,000.

5.48. Ties removed.

6.48. Reds, 7,456,000.

Experiment 43, January 13, 1915. Dog. 6.9 kilo.

11.46. Chloretone intraperitoneally. 2.5 gm.

12.20. Reds, 8,808,000.

12.55. Both jugular veins and carotid arteries prepared, left exposed but not tied.

1.45. Reds, 9,224,000.

2.43. Reds, 9,456,000.

4.22. Reds, 10,688,000.

5.24. Reds, 11,296,000.

Experiment 44, January 14, 1915. Dog, 4.5 kilo.

11.45. Chloretone 1.5 gm. intraperitoneally.

11.57. Reds, 9,184,000.

Control experiment. No operation done.

2.10. Reds, 8,952,000.

6.15. Reds, 7,544,000.

Dog still asleep, put away in a warm place.

January 15. Dog well and asleep.

6.15 p.m. Reds, 8,256,000.

January 16. 12.27. Reds, 8,664,000. Dog still asleep.

12.34. Left vagus nerve exposed, tied and cut, stimulated centrally for two minutes.

1.05. Reds, 9,600,000.

1.14-1.16. Stimulated again.

2.13. Reds, 9,128,000.

2.35-2.40. Right vagus nerve exposed, cut and the central end stimulated two and one-half minutes. Immediately afterwards stimulated the left vagus again for two and one-half minutes.

3.35. Reds, 8,856,000.

5.27. Reds, 8,472,000.

Besides these experiments, it had been noticed, in operations on the thoracic duct in which the operation was prolonged, but the duct not cut, that after this operation the count was exceptionally high, in one dog increasing from 10,240,000 to 11,616,000 in a two hour operation, and in another being 11,808,000 after an operation of about the same duration.

On account of the close proximity of the vagus and sympathetic nerves to the field of operation in these experiments, irritation of them was of course thought of as a possible factor in the increased counts.

The sheath of the vagus sympathetic nerves was exposed, both nerves cut and stimulated centrally together. The result as shown in experiments 44 and 45 is a slight increase in the red count but nothing in comparison with some other experiments in which no direct stimulation of the nerve took place.

Experiment 45, January 19, 1915. Dog, 7.4 kilo.

11.15. Chloretone 2.5 gm. intraperitoneally.

4.44. Reds, 8,573,000.

4.47. Left vagus exposed and stimulated constantly with a weak current for one hour.

5.42. Reds, 9,143,000.

6.05. Reds, 9,053,000.

(C) *Emotional stimuli.* Besides in experiments 13, 14 and 15 we have seen that the red count is greatly influenced by emotional changes.

We see then that various forms of nerve stimulation can give rise to an increase in the number of red cells. It would of course be possible to follow out the effect of stimulation of the different parts of the brain, cord, etc., and locate the nerves which when stimulated will affect the number of red cells. But this would be a tedious task and one needing endless control experiments. Let us consider then the stimuli which occur under physiological conditions which are capable of increasing the red count, and from a study of these see if we can gain any information as to how the body normally reacts to physiological stimuli, and maintains a red count capable of supplying the body with the sufficient amount of oxygen.

Such stimuli are various forms of asphyxia, such as those produced by changes in climate, exercise, with its call for more oxygen, emotional stimuli such as anger and fear.

(3) *The part played by the adrenals*

Each of these conditions has been shown to do two things, that is, to stimulate the splanchnic nerves, and to increase the output of epinephrin from the adrenal gland (69).

We have seen that the injection of epinephrin is one of the most certain means of effectually increasing the red count. One might assume then that the oxygen content of the blood is very simply regulated. When this quantity falls below normal the resulting asphyxia stimulates the splanchnic nerves, which in turn cause the adrenals to secrete epinephrin, which is carried to the liver, and there causes changes which result in an increase of the red corpuscle and haemoglobin content of the blood. These changes soon allow the body to acquire the necessary amount of oxygen, and when this quantity has reached the normal again, stimulation of the splanchnics ceases, epinephrin is no longer excreted, the liver stops producing cor-

puscles, and the red count falls again. Such a mechanism may be thought of as acting continually only by changes which are entirely overlooked. It is only when there is some exceptional call for more oxygen that the body responds by changes which we notice.

But it is certain that this is not all that there is to this mechanism. In fright for example the count as we have seen may suddenly increase tremendously. There has been no increased consumption of oxygen at all proportional to the increase in number of red cells. Our assumed mechanism must then be sensitive to other stimuli than lack of oxygen, in this case an emotional stimulus. But it has been shown that this stimulus also causes an increased output of epinephrin, so that we cannot rule out the possibility of its acting through the same mechanism as asphyxia.

The question naturally arises then as to the actual part played by these glands in the regulation of the red count, and as to whether these same stimuli will produce an increase in the red cells after extirpation of the glands.

(a) *The effect of adrenal removal.* The theory that epinephrin secreted from the adrenals is the sole agent by which the red count may be increased in the body receives a hard blow when one learns that removal of the adrenals produces, after six to twenty-four hours, an increase of as much as one hundred per cent in the number of red cells (70). How is it then that the body with no adrenal glands can increase the red count even more than it is increased under most known stimuli? This is a question which cannot at present be answered, but that this fact excludes the possibility of the adrenal glands playing the part which we have just laid out for them in regulating the red corpuscle content of the blood is not at all necessary. We have spoken many times of the different possible ways in which the number of red corpuscles could be increased in number. We have seen that the loss of fluid from the blood is one of these. It is simple to suppose that removal from the blood of a substance which is so intimately connected with the vascular tone, permeability of the vessel walls, etc., as epinephrin has been

shown to be might be followed by changes in the fluid content of the blood which could give rise to a very marked relative polycythaemia.

(1) *Fright after adrenal removal.* To investigate the effect of emotional stimuli after the removal of the adrenals, two cats were etherized at the same time, and the adrenals exposed in both. In one the wounds were merely left open while the glands were being removed from the other. Both cats were then sewed up at the same time, and allowed to fully recover from the effect of the ether.

They were then frightened by a dog, and counts taken before and after this fright.

The cat in which the adrenals had been removed had no increase in the red count, and in fact it fell a little.

The cat in which the adrenals were still intact had a distinct increase in the red count.

It is also of interest to note the initial counts in both cats. In the cat without the adrenals the initial count is very low for a cat tied down without ether. The other cat in which the adrenals are still present had the customary high initial count.

Experiments 46 and 47, June 7, 1915. Cats.

<i>Control Cat.</i>	<i>Adrenals removed.</i>
10.45. Ether.	10.50. Ether.
11.02. Adrenals exposed.	11.12. Operation begun.
Wounds left open.	11.54. Adrenals removed.
11.59. Cat sewed up. Ether discontinued. Put in cage.	Ether discontinued. Cat put in cage.
12.37. Both cats out of ether sitting up normally.	
2.16 Reds, 11,024,000.	2.53. Reds, 9,504,000.
2.32. Reds after frightening by a dog, 11,680,000.	2.56. Scared by dog.
	3.10. Reds, 9,136,000.
Both cats killed with ether.	

(2) *The effect of asphyxia, pituitrin, and epinephrin after the removal of the adrenals.* As stimulation of nerves is such an unsatisfactory method of increasing the red count, and as the experiment in which a cat was frightened after removing the adrenals is open to the criticism that the animal had not com-

pletely recovered from the anaesthesia, other methods were sought by which an increase in the red count could be obtained while the animal was under the influence of an anaesthetic. For this purpose asphyxia was again used, and a new substance, pituitrin, which could be intravenously injected.

As we have seen in previous experiments asphyxiating an animal is almost certainly followed by an increase in the red count.

Control experiments were done to ascertain the degree of polycythaemia after the injection of pituitrin. One-half hour after the injection of this substance in two dogs, the counts rose 700,000 and 500,000 respectively.

The adrenals were then removed in two other dogs, counts taken and the dogs asphyxiated under ether for fifteen or twenty minutes. This caused *no increase* in the number of red cells.

Pituitrin was then injected intravenously into both dogs. One-half hour after the injection there was *no increase* in the red count.

It appeared then that removal of the adrenals prevented the customary increase in the number of red cells after both the asphyxia and the injection of pituitrin.

In order to show that the animal is capable of increasing the number of red cells per unit volume of blood on the application of the proper stimulus, after removal of the adrenals, injections of epinephrin were made in the usual dose of 0.9 mg. per kilo intravenously.

It will be seen that in both animals the injection of epinephrin was immediately followed by the usual rise in the number of red cells.

Experiments 48 and 49. Dogs, 13.1 and 7.1 kilo, respectively.

Ether.

Counts 7,200,000 and 7,144,000 before injection.

Injected 2 and 3 ccm. Park Davis Pituitrin intravenously.

Counts. 7,896,000 and 7,656,000, respectively, one-half hour after injection.

Experiment 50, June 2, 1915. Dog, 6.5 kilo.

11.10. Ether.

- 1.00. Both adrenals out.
- 1.50. Reds, 7,360,000.
- 1.53-2.12. Asphyxiated.
- 2.12. Reds, 7,200,000.
- 2.23. Reds, 7,456,000.
- Plenty of air now given.
- 3.14. Reds, 7,096,000.
- 3.18. Pituitrin 2 ccm.
- 3.33. Reds, 7,264,000.
- 3.53. Reds, 7,280,000.
- 3.55. Epinephrin 0.9 mg. per kilo, intravenously.
- 4.25. Reds, 9,424,000.
- Experiment 51, June 3, 1915. Dog, 5.8 kilo.*
- 2.28. Ether.
- 3.27. Both adrenals out.
- 3.47. Reds, 7,096,000.
- 3.47-4.11. Asphyxiated.
- 4.17. Reds, 7,144,000.
- 4.22. Pituitrin 3 ccm. intravenously.
- 4.52. Reds, 6,664,000.
- 5.04. Epinephrin 0.9 mg. per kilo, intravenously.
- 4.23. Reds, 8,640,000.

We have then a certain amount of evidence to show that the adrenals play a part in the conditions of asphyxia, pituitrin injection, and fright. After their removal, none of these conditions produce the usual increase in the number of red cells.

That the adrenals are necessary for the proper regulation of the red corpuscle content of the blood is also shown by the great changes produced in the red count some time after their removal.

Sufficient data are not yet on hand to determine if secretion of epinephrin by the adrenals is the only factor capable under physiological conditions of increasing the red count.

DISCUSSION

Let us now go over the results of these experiments, correlating them with the findings of other investigators, and see what is known about the changes in the number of red corpuscles per unit volume of blood.

In the first place we see that the number of red cells per unit volume of blood is *not* a constant, under physiological conditions, as is so generally supposed by those who have not paid especial attention to this problem. But instead, it is constantly oscillating, under such conditions as changes in the atmospheric pressure, exercise, and, as I have shown, in emotional conditions such as fright and anger.

When we investigate the various factors which will cause an increase in the number of red cells, we find that they are very many. Any condition, physiological or pathological, which causes a decrease in the amount of oxygen in the blood may increase the number of red corpuscles.

Stimulation of certain nerves, either electrically or by other forms of stimulation such as fear, reflexly causes an increase in the number of red cells. Besides this the injection of various chemical substances, some of which are physiologically found in the body, as epinephrin, pituitrin, carbon dioxide, etc., and of others which are entirely foreign to it, will cause a marked polycythaemia.

We wish then to know in the first place if there is an actual increase in the number of red corpuscles, or whether this increase is merely relative. We also wish to know *where* this increase takes place, and how it is brought about, and finally on account of the apparent regulation of the number of red corpuscles per unit volume of blood we wish to know what the regulation of this mechanism is, and what is its function.

We have seen that in one type of polycythaemia, namely that produced by epinephrin injection, the increase in number of red cells is due entirely to the part played by the liver. The obstruction of the arterial blood supply to the liver by ligature will exclude the usual increase in red cells after epinephrin injection, and removal of this obstruction with a return to the normal arterial blood supply, through the hepatic artery, will give the customary increase in red count. It has been shown also that it was possible to produce polycythaemia by the injection of epinephrin after the removal of the spleen, the entire intestine and its mesentery, the omentum and the pancreas.

Furthermore it has been shown by exclusion that in acute epinephrin polycythaemia the bone marrow, muscles, lungs, kidneys and skin play no part.

When we consider how it is that the liver increases the number of red cells, we know that it must be either by a new production of red corpuscles, a splitting of these, loss of fluid from the blood, or a better distribution of corpuscles in the general circulation.

It has been shown that this increase in red cells is due in part to loss of fluid from the blood, but not entirely to this; that there are corpuscles present in the circulating blood during acute polycythaemia which are different from those present before its production. This is shown by their relative decrease in size, and by their reduced percentage haemoglobin content.

Signs of new red cell formation, such as the presence of nucleated red cells, a difference in the fragility of these corpuscles, or an increased metabolism of these cells as shown by an increased reduction, are all absent.

An increase in the number of red cells entirely out of proportion to the increase in haemoglobin percentage, and in rare instances where no increase in the haemoglobin content is found, but where the red cells are increased in number would favor the view that there is a splitting of the red cells.

As regards the presence of a reservoir in which red cells are normally packed away, and from which they may be driven out, it has been shown that if there is such a reservoir it must be in the liver. No experimental evidence for or against such a reservoir has been found.

Massage of the liver itself does not immediately force the red cells into the circulation. It is probable then that the increased counts observed after massage of the abdomen are due to a reflex stimulation of the adrenal glands and secondary action on the intestinal walls, rather than to an actual mechanical driving of corpuscles from reservoirs in the abdomen.

The liver appears then to have the following functions.

(a) The ability to decrease the plasma volume after epinephrin injection. For this change to take place the arterial

blood supply to the liver must be intact. Interference with the portal circulation has no influence on this action.

(b) The ability to cause the presence in the generally circulating blood after epinephrin injection of red cells of decreased average volume, and with a decreased percentage haemoglobin content, which give no signs of young cells by the presence of nucleated red cells, increased fragility, or increased metabolism of the cells themselves, as shown by an increased rate of reduction.

(c) By these two methods the number of red corpuscles per unit volume of blood is increased, also the haemoglobin content, but not to the same extent as the number of red cells. The oxygen carrying capacity of the blood is increased but only in proportion to the increase in haemoglobin. The surface of the haemoglobin is however greatly increased, and the blood is better equipped to take up more oxygen per unit of time.

As regards a mechanism by which the red count may be regulated, it has been shown that epinephrin is one of the most effective stimuli capable of increasing the red count; that all of the main physiological and pathological conditions, which might call for an increase in the oxygen content of the blood, stimulate the adrenal glands, and cause an increased output of epinephrin.

Theoretically a mechanism operating by any decrease below the normal oxygen content of the blood, stimulating the splanchnic nerves and acting on the adrenal glands causing an increased output of epinephrin, and a direct action of this substance on the liver, and thus increasing the red cells, is possible. The blood after obtaining the necessary content of oxygen would then cease to stimulate the adrenals, the epinephrin output would fall, and consequently the stimulation of the liver would cease, and the count would then decrease until the oxygen content became sufficiently low again to cause adrenal stimulation, and an increase in the number of red cells.

Evidence for this theory consists in absence of polycythaemia after fright, asphyxia, and pituitrin injection in animals in which the adrenals have been removed; also by the fact that removal of the adrenals completely upsets the control of the red count in a few hours.

We might conclude then that the adrenals may play a rôle in controlling the red count under ordinary conditions of life. That the body does not necessarily have to wait until the oxygen content of the blood is below normal before responding by an increase in the count is well shown by the great increase in the number of red cells during fright. We have here before there is any lack of oxygen, or real asphyxia, a sudden and very great preparation on the part of the body to meet eventualities. The blood is equipped, not only with an increased amount of haemoglobin, and thus a greater oxygen capacity, but the surface of this haemoglobin is increased by a rise in the number of red corpuscles. The body is then furnished with blood of maximum efficiency to meet very variable circumstances.

That it is the oxygen content of the blood, and not its oxygen capacity, or the number of red cells per unit volume of blood, which the body attempts to maintain constant, is, I believe, the object of this mechanism.

SUMMARY

(1) The red corpuscle content of the blood is a constantly varying quantity under physiological conditions.

(2) Certain new means have been found by which the red count may be rapidly and greatly increased.

(a) Lung emboli produced by the injection of corpuscles hardened with formaldehyde, of an inert powder as lycopodium, or of oil.

(b) Emotional stimuli, as fright and rage.

(3) The polycythaemia produced by the injection of epinephrin is due to the action of the *liver alone*.

(4) Ligature of the arterial blood supply to the liver excludes the production of polycythaemia after the injection of epinephrin.

(5) Later release of this ligature allows the customary increase in number of red cells to take place, without the further injection of epinephrin.

(6) The liver effects this increase in number of red cells by

(a) A decrease in plasma volume, not sufficient to account for the entire increase in the number of red cells however.

(b) By bringing into the circulation red cells which were not present before the production of polycythaemia as shown by

(α) Their reduced size.

(β) Their reduced percentage haemoglobin content.

(7) These cells give none of the usual reactions of young cells. There is an absence of nucleated red cells, no change in the fragility of the corpuscles, and no increased metabolism of the red cells themselves, as shown by an increased rate of reduction.

(8) It is concluded that there is a mechanism for the regulation of the red corpuscles content of the blood.

(9) That the regulatory mechanism is under nervous control, reacting to lack of oxygen as a stimulus.

(10) That the adrenal glands play a part in this mechanism.

(11) That the *liver* is the organ which supplies the body with red cells to meet its acute demands.

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THE SEAT OF THE EMETIC ACTION OF VARIOUS DRUGS

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Nausea and vomiting are probably among the commonest of the undesired side actions produced by a considerable number of our more important drugs. In order to use a drug to the best advantage an understanding of the mechanism of the production of its side actions is quite as essential as an understanding of its principle actions. It is the purpose of the present paper to record the results of approximately 500 experiments, including those previously reported, made to determine the probable seat of the emetic action of some of the more important drugs, and of others of less importance, but which have a very marked tendency to cause emesis.

It has been previously shown (1), (2), (3) that the vomiting produced by therapeutic doses of the digitalis bodies is probably wholly due to a direct action on the vomiting center, resulting only after the absorption of a certain minimal quantity of the drug into the circulation. It is obvious, therefore, that this action cannot be prevented except by a proper limitation of the amount of digitalis administered, or by the administration of some narcotic to depress the vomiting center. The latter is of course an undesirable means of control, but the explanation of the mechanism renders the avoidance of this side action easier of accomplishment and also establishes it as a sign of the absorption of the drug.

In an earlier communication (4) the evidence which is required to establish the fact that an emetic, such as apomorphine, acts directly upon the vomiting center was reviewed. The evidence

of greatest value in the proof of such an action is: 1. That the drug produces emesis in a smaller dose by intravenous injection than by oral administration. 2. That it acts more promptly when given by vein than by mouth. 3. To this we added a third observation, namely, that it produces the typical phenomena associated with nausea and vomiting when given to a dog from which the alimentary canal from the cardiac end of the oesophagus to within a short distance of the anus has been removed. Other methods for the determination of the seat of emetic action have been employed, but they are either too difficult to be readily applied, or are fraught with serious objections which detract largely from their value. Of these only one deserves special mention—the method devised by Thumas (5)—particularly because it bears directly upon the question of the location and very existence of a vomiting center.

Whether or not there is an actual anatomical nerve center presiding solely over the act of vomiting is still a matter of dispute among physiologists, but the evidence seems overwhelming for the belief that there is some more or less specialized central nervous mechanism, possibly merely of a coördinating nature, which does control the act of emesis. It is in the latter sense that we use the term, "vomiting center" in the following pages.

In the present investigation we have limited our observations to the determination of the approximate minimal emetic doses of each of the drugs for dogs by intravenous and oral administration (in a few instances we have also roughly determined this for intramuscular administration); to the determination of the rapidity of action by these channels of administration; and of the ability of the several drugs to cause the typical phenomena of the vomiting act when given to dogs after the removal of the alimentary canal. Certain other actions of the drugs were also observed in the course of these experiments, but these will not be detailed unless they have some bearing upon the problem in hand.

Morphine. The production of nausea is a very common side action of this drug, and vomiting not infrequently follows its

administration, irrespective of how given. The known central actions of morphine, and the virtual absence of all local action, except an ill understood one associated with the production of constipation, would lead to the belief that it caused nausea and vomiting also through a direct action upon the medulla. While this is the concensus of opinion, it has been suggested that these

TABLE I

Morphine Sulphate

	MGM. PER KILO	EFFECT*	TIME IN MINUTES	REMARKS
Vein.....	0.050	N?	2½	
	0.075	N	2	
	0.085	N	1¾	
	0.100	N	1¼	
	0.100	N	1¾	
	0.100	E	2	
	0.150	N	1½	
	0.150	N	2	
	0.150	E	3	
	0.200	0	0	Depressed—analgesia
	0.200	0	0	Depressed
	0.200	E	2½	} Slight depression in all
	0.200	E	1½	
	0.250	E	2	
	0.250	E	3	
Oral.....	1.000	0	0	
	2.000	N	3	
	4.000	N	5½	
	6.000	N	4	
	10.000	N	4	
	12.500	0	0	
	15.000	N	2	
	15.000	E	9	
	15.000	E	5½	

* In all of the tables the letters given in this column are used as follows: N means nausea, E means emesis, R means retching.

effects might be due to the excretion of the drug into the stomach after its subcutaneous administration, or to its action in producing a more or less lasting spasm of the pylorus. Direct evidence as to the seat of its emetic action seemed, therefore, desirable.

Table 1 gives a brief résumé of the experiments to determine the minimal emetic doses and the rapidity of action by intravenous, intramuscular, and oral administration. While doses of 0.075 mgm. per kilo of body weight and over produced nausea when given intravenously, the smallest emetic dose was 0.1 mgm. per kilo. This dose produced emesis in only one of three dogs, and the same was true of a dose of 0.15 mgm. per kilo. A dose of 0.2 mgm. per kilo caused emesis twice in four administrations, the other two failing on account of the prompt production of depression of a slight grade. The minimum emetic dose by vein can probably be stated as 0.1 mgm. per kilo. When the drug was given intramuscularly the smallest emetic dose was found to be 0.6 mgm. per kilo, while it required 15.0 mgm. per kilo to produce the same effect when the drug was administered orally. From these figures it is seen that the minimal emetic dose by intramuscular injection is six times that by vein, and that for oral administration 150 times as great as by vein.

The average time from injection to the appearance of emesis after intravenous administration in six instances was a little over $2\frac{1}{4}$ minutes. That by intramuscular administration was only slightly longer, while the time for the appearance of vomiting after oral doses was considerably greater in both instances.

The striking difference between the minimal dose by vein and that by mouth is analogous to that seen with apomorphine, in which the oral dose was over one hundred times the intravenous. This, together with the longer time required for emesis after oral administration, at once suggests that the action is purely central. Evisceration experiments were next undertaken to test this view. At the time that the first of these were performed we had not determined the absolute minimal vein dose, but then thought this to be about 0.3 mgm. per kilo. We cite a protocol of this first series:

September 10, 1914. Dog, female, 4.82 kilo.
10.35 a.m. Begin chloroform.
10.48 Chloroform withdrawn.
10.53. Operation completed, animal released.

11.19. Morphine 0.3 mgm. x kgm. by femoral vein. No emesis or nausea; animal depressed.

11.54. Apomorphine 2.0 mgm. total intramuscularly. No emesis or nausea.

In this and all other evisceration experiments the animal was chloroformed to death at once upon its completion.

Three experiments of this type were performed, all with negative results so far as the production of vomiting was concerned. We were at a loss to account for the fact that all of the eviscerated dogs showed depression so promptly after such small doses and that all failed to vomit. Two more experiments were then performed and 2.5 mgm. and 6.0 mgm. per kilo respectively of morphine sulphate were given intramuscularly, but the results were the same as before. Subsequent experiments with normal dogs showed that the minimal emetic dose for intravenous administration was smaller, and that doses of over 0.2 mgm. per kilo usually produced some depression even in normal animals. We then repeated the evisceration experiments on two dogs using doses of 0.15 mgm. per kilo in each case. Both animals showed the phenomena of emesis in typical form after $3\frac{1}{2}$ and $4\frac{1}{2}$ minutes respectively. One of the protocols is appended.

March 19, 1915. Dog, female, 4.8 kilo.

10.10 a.m. Begin chloroform.

10.18. Chloroform withdrawn.

10.22. Operation completed: animal released.

11.08:30. Morphine 0.15 mgm. x kgm. into ear vein.

11.09. Nausea.

11.12. Emesis.

From these experiments we may conclude that the nausea and vomiting resulting from morphine is due to its action upon the vomiting center and not to any local action in the alimentary canal.

Pantopium. This is a proprietary preparation said to be composed of all of the opium alkaloids in their natural proportions in the form of their hydrochlorides. It is claimed for it that it has all of the virtues of opium or morphine with certain valu-

able advantages, among which are that it is less likely to produce nausea and vomiting in effective doses. It was, therefore thought advisable to test this point on dogs. Table II gives the doses administered and their effects, and the times after the administrations before the onset of nausea or emesis. Pantopium is said by the manufacturers to contain 50 per cent of morphine, and from this the amount of morphine contained in each of the several doses given was calculated and is stated in the fourth column of the table. A comparison of the doses in this column which were followed by emesis with those in the table for morphine sulphate (Table I) shows that pantopium is probably actually slightly more actively emetic than is to be accounted for by the morphine alone which it contains. It should be

TABLE II
Pantopium

	MGM. PER KILO	EFFECT	TIME IN MINUTES	MORPHINE CONTENT MGM. PER KILO	REMARKS
Vein.....	0.25	N	1 $\frac{3}{4}$	0.125	
	0.25	N	1	0.125	
	0.25	N	2	0.125	
	0.30	N	1 $\frac{1}{4}$	0.150	
	0.30	N	1	0.150	Slight depression
	0.30	E	1 $\frac{3}{4}$	0.150	
	0.30	E	1	0.150	
	0.30	E	1 $\frac{3}{4}$	0.150	
	0.30	E	2 $\frac{3}{4}$	0.150	
	0.50	R	2 $\frac{1}{2}$	0.250	Depressed

added that there was no evidence that it was any more active than morphine in the production of depression or of analgesia. No evisceration experiment was performed with this preparation as it was obvious that the mechanism of its emetic action was the same as that of morphine.

Narcophin. Another proprietary preparation of opium which has been brought forth with many claims of therapeutic advantage over opium and morphine. The statement has been frequently reiterated that this compound is decidedly less actively emetic than morphine. It is said to be composed of equal parts by weight of morphine, narcotine and meconic acid. The

results obtained from its administration to dogs are recorded in Table III. Here again we have included for ready comparison the calculated amounts of morphine contained in the doses given. The smallest dose of narcophin which produced emesis when given intravenously was 0.15 mgm. per kilo, which is equivalent to 0.05 mgm. of morphine. All doses greater than this produced emesis without a single failure. As seen in Table I, emesis resulted from morphine sulphate only once with a dose of less than 0.15 mgm. per kilo. The one smaller dose was 0.1 mgm. per kilo, or twice that for the morphine contained in narcophin.

TABLE III
Narcophin

	MGM. PER KILO	EFFECT	TIME IN MINUTES	MORPHINE CONTENT MG. PER KILO
Vein.....	0.10	0	0	0.033
	0.10	0	0	0.033
	0.15	N	1	0.050
	0.15	N	1½	0.050
	0.15	E	2	0.050
	0.15	E	2¾	0.050
	0.20	E	2½	0.066
	0.30	E	1¼	0.100
	0.40	E	2	0.130
	0.50	E	2½	0.160
Oral.....	2.50	0	0	0.830
	4.00	N	10	1.330
	4.00	N	4	1.330
	4.50	E	12½	1.500
	4.50	E	4½	1.500
	5.00	E	5½	1.660

From a comparison of these two tables narcophin also appears to be more certainly emetic than morphine sulphate. In no instance did it cause appreciable depression or analgesia in the doses given, although nearly twice the minimal emetic dose was used in one animal.

Narcophin was also given orally to a few dogs and the minimal emetic dose thus determined was 4.5 mgm. per kilo, containing 1.5 mgm. of morphine. With morphine sulphate we saw that the smallest oral emetic dose was 15.0 mgm. per kilo. This is

ten times as great as the dose of morphine which was effective when given in the form of narcophin.

These two series of experiments leave no doubt that, for dogs at least, narcophin is much more actively emetic than is morphine in the form of the sulphate, rather than less so as claimed. It is also obvious that it is decidedly more emetic in proportion to its analgesic and hypnotic actions than is morphine.

In addition to these observations we performed two evisceration experiments with narcophin. The protocol of one is appended:

March 19, 1915. Dog, female, 6.8 kilo.

11.45 a.m. Begin chloroform.

11.53. Chloroform withdrawn.

11.55. Operation completed: animal released.

12.23:10 p.m. Narcophin 0.2 mgm. x kgm. into ear vein.

12.23:50. Nausea.

12.24:55. Emesis.

In the other experiment a small amount of the first injection was lost in the subcutaneous tissues through struggling of the dog. The dose was, therefore, repeated after an interval of 15 minutes but failed to induce emesis although the dog retched once or twice and seemed nauseated. There was decided evidence of depression in this animal coming on within six minutes after the second dose.

Since narcophin is said to consist of equal parts of narcotine, morphine and meconic acid, and since the latter is not an active pharmacologic agent, we thought it worth while to test a mixture of equal parts by weight of narcotine (dissolved with the aid of a trace of dilute HCl) and morphine sulphate with respect to its emetic activity. The results of these experiments are set forth in Table IV. Although this mixture was not always followed by vomiting in the doses used, the three instances in which emesis resulted were all in animals receiving a dose of morphine smaller than the smallest average emetic dose of the sulphate alone. This would seem to show that the mere mixture of these two substances led to some increase in the emetic activ-

ity of the morphine contained. Observations made on narcotine alone showed it to be without emetic action, but to have a considerable stimulant action on the respiratory mechanism, often producing marked panting. Large doses produced convulsions of tonic and clonic types combined. It seems possible that the increase in the emetic action of morphine when mixed with narcotine, or when combined with it as in narcophin, might be explained on the supposition that the narcotine rendered the vomiting center more susceptible to the influence of morphine than is normally the case. This would be quite analogous to

TABLE IV
Narcotine + Morphine—equal parts

	OF EACH, MGM. PER KILO	EFFECT	TIME IN MINUTES	REMARKS
Vein.....	0.035	N	1 $\frac{3}{4}$	
	0.040	N	1 $\frac{1}{4}$	Defecation
	0.050	N	1 $\frac{1}{2}$	
	0.050	N	1	Panting
	0.050	N	1	
	0.050	E	2 $\frac{1}{2}$	Slight depression
	0.075	N	1	
	0.075	N	1	
	0.080	N	1 $\frac{3}{4}$	
	0.100	N	2	Slight depression
	0.100	N	1 $\frac{1}{4}$	
	0.100	E	2	
	0.100	E	3	Panting

the synergistic actions of cocaine and epinephrine upon the vasoconstrictor endings.

The ipecac alkaloids—Emetine and cephaeline. Although ipecac has long been used as an emetic in the form of one or another galenical preparation, and in spite of the fact that its alkaloids have long been known and much studied, there is yet no definite agreement as to the seat of their emetic action. The commonly accepted view is that both emetine and cephaeline are reflex emetics, acting solely through their irritant effects on the gastrointestinal mucous membrane. This is supported by the known irritant properties of these alkaloids when applied to the conjunctiva, the preputial membrane, or even to the skin; by the

fact that emesis is said not to follow more rapidly after the subcutaneous or intravenous injection of either alkaloid than after its oral administration; by the frequent post mortem evidence of hemorrhagic inflammatory lesions in the upper intestinal tract following large doses of either alkaloid irrespective of the channel of administration; by the statement that the drug is present in the vomitus after subcutaneous or intravenous administration; and lastly, by the fact that vomiting did not result from the administration of emetine to cats or dogs after vagotomy in the hands of some investigators.

Only one observer, so far as we are aware, has offered any convincing evidence in favor of emetine's having a direct action upon the vomiting center. This is Thumas (5) who found that the direct application of small amounts of dilute solutions of emetine to the region of the medulla in which he believed the center to lie produced emesis much as did apomorphine, and that emetine was the only drug other than apomorphine which acted thus. A third group of pharmacologists bridge the gap and state that the drug may have both reflex and central actions.

Before proceeding to the results of the present series of experiments a few words should be said regarding the validity of the evidence advanced in favor of the drug's action being purely local and reflex. In the first place, as we have already said in another paper (1) the fact that a drug is irritant to one of the superficial mucous membranes is not necessarily proof that it is also irritant to the mucosa of the alimentary canal. Dückworth (6) who believed in the local reflex emetic action of emetine, called attention to the fact that after small doses, but such as produced emesis, there was often no demonstrable inflammation of this region, although such was constantly present after large doses, its intensity being proportionate to the time that the animal survived the administration. Further, Podwyssotzki (7) suggested that this action might be analogous to that of arsenic upon the vasomotor mechanism, particularly as it occurred after sufficient doses irrespective of the channel of administration. If the drug caused emesis solely through its local

irritant action, one would certainly expect the action to result more rapidly from oral than from intravenous or subcutaneous administration. But most observers state that there is no difference in the times before emesis appears after the several modes of administration. We will have more to say on this point when we come to present our own observations. The statement that the drug is present in the vomitus has been made by several observers, including d'Ornellas (8) who produced emesis in pigeons with the vomitus of animals which had received the drug subcutaneously, and Foulkrod (9) who claimed to have found the drug in the vomitus by means of a chemical test. On the other hand, more observers have failed to find the drug in the vomitus than have succeeded. Among whom may be mentioned Podwyssotzki (7) and Lowin, (10)¹ the latter working in Kobert's laboratory. Although several observers failed to secure emesis from emetine after vagotomy in cats or dogs, their experiments cannot be regarded as conclusive, for, in most instances, it is evident that they kept their animals restrained upon their backs—a position which materially reduces the occurrence of emesis even in the intact animal after apomorphine.

The preparations which we used were from two sources. One was a highly purified specimen of emetine hydrochloride which has recently been placed upon the market. The remainder were specimens of purified emetine, cephaeline, and of the isolated, mixed, purified alkaloids in the proportions in which they exist in ipecac. In addition to these we also used a preparation of the total ipecac alkaloids precipitated by means of hydrous aluminum silicate (Lloyd's reagent). All of the last named preparations were generously supplied to us by Prof. John Uri Lloyd, to whom we wish to acknowledge our indebtedness. In fact, had it not been for the kind coöperation of Professor Lloyd we probably should have been unable to have employed cephaeline or the total isolated alkaloids, for the present war had then closed the European drug markets and

¹ This paper of Lowin's reviews much of the more important literature on ipecac and its alkaloids, and contains a bibliography covering nearly everything of value published up to the date of its preparation.

TABLE V
Emetine hydrochloride (commerical)

	MGM. PER KILO	EFFECT	TIME IN MINUTES
Vein*.....	2.0	N	18
	3.0	0	0
	3.0	N	13
	3.0	E	52½
	4.0	N	12
	5.0	E	16½
	7.5	E	14½
Oral†.....	1.5	0	0
	1.5	0	0
	2.0	N	41
	2.0	E	41
	2.0	E	54

* Additional doses by vein of 1.0, 2.0, and 2.0 mgm. per kilo were given without effect.

† Additional doses were given orally of 3.0, 3.0, 4.0, 5.0, 5.0, 7.5, 10.0, 12.5, mgm. per kilo, all of which caused vomiting in from 12 to 51 minutes, except one of 5.0 which produced no symptoms.

TABLE VI
Emetine (sulphate)—Lloyd

	MGM. PER KILO	EFFECT	TIME IN MINUTES
Vein*.....	2.0	0	0
	2.0	0	0
	3.0	0	0
	3.0	E	16
	3.0	E	20
Oral†.....	1.0	0	0
	1.5	0	0
	2.0	N	43
	3.0	0	0
	3.0	E	17
	3.0	E	58

* Doses of 4.0, 5.0, and 5.0 mgm. per kilo were given, all producing emesis in from 13½ to 22 minutes.

† Doses of 4.0, 5.0, 5.0, 7.5, 10.0, and 15.0 mgm. per kilo were given, all producing emesis in from 27 to 98 minutes, except the dose of 10.0 mgm. which produced depression and nausea only.

TABLE VII
Cephaeline—Lloyd (Sulphate)

	MGM. PER KILO	EFFECT	TIME IN MINUTES
Vein*.....	1.00	0	.0
	1.00	N	27
	1.00	N	17
	1.00	E	6
	1.50	E	43
Oral†.....	0.50	E	38
	0.75	0	0
	0.75	E	38
	1.00	0	0
	1.00	E	86
	1.00	E	90

* Doses of 1.5, 2.0, 2.0, 5.0, and 8.0 mgm. per kilo were given, all of which produced emesis in from 13 to 34 minutes.

† Doses of 1.5, 2.0, 2.0, 3.0, 5.0, 7.5, 7.5, 10.0, 10.0, 15.0, 17.5, and 20.0 mgm. per kilo were given, all producing emesis in from 22 to 68 minutes, except one of 10.0 and the dose of 17.5 mgm. The doses of 15.0, 17.5, and 20.0 mgm. per kilo were fatal.

TABLE VIII
Total ipecac alkaloids—Lloyd (Sulphate)

	MGM. PER KILO	EFFECT	TIME IN MINUTES
Vein.*.....	1.00	0	0
	1.00	0	0
	1.00	N	8
	1.50	E	13
	2.00	N	23
	2.00	E	14½
Oral†.....	0.50	0	0
	0.50	0	0
	0.75	0	0
	0.75	E	41
	0.75	E	17

* Doses of 2.5, 3.0, 3.0, and 4.0 mgm. per kilo were given, all producing emesis in 13 to 64 minutes.

† Doses of 1.0, 1.0, 2.0, 3.0, 4.0, 4.0, 7.5, 10.0 and 15.0 mgm. per kilo were given, all producing emesis in from 20 to 70 minutes, except one of 4.0 and the 7.5 mgm., which cause nausea only. The doses of 10.0 and 15.0 mgm. were fatal.

we were unable to procure any cephaeline from American dealers².

In Tables V, VI, VII and VIII there are recorded the doses given to dogs to determine the minimal emetic dose for each preparation by both oral and intravenous administration. It will be seen that the two preparations of emetine (Lloyd's and the commercial one) were of the same activity. We may, therefore, consider the figures of these two tables together. The smallest emetic dose by intravenous injection was 3.0 mgm. per kilo, and 2.0 mgm. per kilo produced nausea in one animal. On the other hand, when given orally 2.0 mgm. per kilo produced emesis in two animals and nausea in two others. From the limited number of experiments with these small doses it would seem that emetine was slightly more actively emetic when introduced directly into the gastro-intestinal tract than when given intravenously. We are inclined to believe that this difference is more apparent than real, for it will be noted that larger doses than 2.0 or 3.0 mgm. per kilo sometimes failed to provoke emesis in both series of administrations—oral and intravenous. A much more striking difference in the actions elicited by the two modes of administration is seen in the matter of the time taken to produce emesis. Regarding nausea and emesis as essentially the same, the 11 experiments with the intravenous administration of emetine which were followed by either or both of these effects gave an average time of $19\frac{1}{2}$ minutes before the onset of symptoms. On the other hand, of the 18 oral administrations (excluding one in which there was marked depression caused by the drug), gave an average time of 41 minutes before the onset of either nausea or vomiting. This difference would seem to more than negative the very slight difference apparent between the sizes of the oral and intravenous doses, and suggests that the delay after oral administration may be due to the time necessary for absorption to take place. It is possible that the somewhat smaller dose required for oral administration may be explained

² The details of the processes by which he isolated and purified these ipecac alkaloids, as well as the alkaloids of lobelia and tobacco, will be published by Professor Lloyd shortly in the American Journal of Pharmacy, to which the reader is referred for further information.

on the assumption that the locally irritant action of the drug when thus brought into direct contact with the gastro-intestinal mucosa may reflexly render the vomiting center somewhat more sensitive than normal. We are not in a position to test the validity of this suggestion, but it would appear to be more or less in line with the work of Head upon the production of hyper-sensitive regions in the central nervous system through the reflex effects of local irritation. In other words, there seems to be a synergism between the local and central actions.

An evisceration experiment was undertaken, the protocol of which follows:

November 24, 1914. Dog, female, 4.8 kilo.

9.50 a.m. Begin chloroform.

10.05. Chloroform withdrawn.

10.06. Operation completed: animal released.

10.46:45. Emetine 7.0 mgm. per kgm. into femoral vein.

10.56. Slight nausea.

11.12. Emetine 3.5 mgm. per kgm. into femoral vein.

11.16. Nausea.

11.35. Emesis.

As this experiment was positive in its results it was not deemed necessary to repeat it upon other animals.

In the case of cephaeline,³ the results obtained were exactly analagous to those already discussed for emetine and the same arguments may be used respecting its seat of action. One difference only appears from a comparison of these tables, namely that cephaeline is more than twice as actively emetic as emetine. This has already been reported by Lowin (10) for animals, and by Wild (11) for man, and we can merely confirm these obser-

³ Both the emetine and the cephaeline obtained from Professor Lloyd were supplied in the form of the isolated alkaloids. From these we prepared our solutions with the aid of dilute sulphuric acid, the excess being neutralized with sodium hydroxide before use. The isolated total alkaloids were sent to us in solution in a slight excess of sulphuric acid, which was similarly neutralized before use. The doses given of these preparations were based upon the weight of the alkaloids, while those of the commercial emetine hydrochloride are stated in terms of weight of this salt. The difference in the molecular weights of the hydrochloride and the base is too slight to have any material effect on the size of the doses given, and this has, therefore, been disregarded.

vations. Two evisceration experiments were performed with cephaeline, the protocol of one only being given here as the second was quite like the first.

March 31, 1915. Mongrel female dog, 5.24 kilo.

10.25 a.m. Begin chloroform.

10.33. Chloroform withdrawn.

10.39. Operation completed: animal released.

11.09. Cephaeline 2.0 mgm. per kilo into ear vein.

11.17. Nausea intense.

11.21. Retching.

11.24:30. Emesis. Repeated at 11.35.

The experiments made with the preparation of the total isolated alkaloids establish about that dose for this preparation which would be expected from a mixture of cephaeline and emetine in approximately the proportions of one part of the former to two of the latter. Subsequent to this observation we learned from Professor Lloyd that there was 34.16 per cent of cephaeline in this preparation, the remainder being mainly emetine, for psychotrine is present in ipecac in traces only. With this preparation, too, the relation between the average times for oral and administrations is roughly the same as that for either emetine or cephaeline. No evisceration experiments were made with this preparation, as each of the alkaloids had given emesis under such circumstances.

From these observations we feel warranted in stating it as our conviction that both emetine and cephaeline are capable of producing nausea and vomiting through a direct action upon the vomiting center and, in view of the almost uniformly more rapid action resulting from intravenous administration as compared with oral, we lean strongly to the belief that their emetic action is probably almost entirely a central one. It is always possible that a drug with some degree of local irritant action may produce emesis in susceptible animals or men by reflex action, but the evidence here brought forth would certainly seem to indicate that such is not the usual mechanism in dogs.

We have also tested "Alcresta ipecac," which is a preparation

of the total alkaloids of ipecac precipitated in combination with hydrous-aluminum silicate. This is insoluble in water and is said to yield its contained alkaloids only in an alkaline medium. It should, therefore, pass the stomach without the liberation of either alkaloid, and hence be devoid of local irritant action there. If the alkaloids were then liberated in the alkaline intestinal fluids emesis might result either from reflexes set up by local irritation in the intestine, or, if absorbed, from central action. The preparation was suspended in distilled water and given orally to dogs in doses ranging from 3.0 to 16.0 mgm. per kilo of the total contained alkaloids. The animals were observed

TABLE IX
Quinine hydrochloride

	MGM. PER KILO	EFFECT	TIME IN MINUTES
Vein.....	25.0	N	13
	50.0	0	0
	50.0	E	16
	50.0	E	12½
	75.0	0	0 Death at once
Oral.....	50.0	N?	3½
	75.0	E	76
	100.0	N	66
	150.0	E	45
	200.0	E	30

for at least three days but failed to show any symptoms whatever of an action of the drug. This might be taken to be evidence in support of the local emetic action of the ipecac alkaloids. It is to be noted, however, that doses of 10.0 mgm. per kilo of the total alkaloids, when given in solution in the form of the sulphates, were fatal while, in the case of the "Alcresta" preparation none of the dogs showed any disturbance whatever. It would seem, then, that the explanation of the want of both emesis and of serious or fatal poisoning following the administration of this preparation is to be found in the slow liberation and absorption of the alkaloids even in the alkaline intestinal fluids.

Quinine. A decided difference is encountered between both the doses and the times for the development of emesis from this drug when the intravenous and oral doses are compared, as shown in Table IX. Vomiting ensued in about the same length of time after intramuscular administration as after intravenous, although the dose required was larger by the former method (75.0 mgm. per kilo). The fact that it took nearly four times as long for emesis to appear after oral administration as after intravenous at once suggests a central action, resulting only after adequate absorption from the alimentary canal. This was confirmed by an evisceration experiment, which is here recorded in brief:

April 1, 1915. Mongrel male dog, 5.86 kilo.

10.14. Begin chloroform.

10.26. Chloroform withdrawn.

10.28. Operation completed; animal released.

11.16. Quinine 25.0 mgm. per kilo into ear vein.

11.21. Quinine 25.0 mgm. per kilo into ear vein.

11.24. Nausea.

11.43:30. Emesis.

Two other eviscerations were made to test the seat of the emetic action of quinine, in each of which the full dose of 50.0 mgm. per kilo was given at once. In one of these convulsions were produced and these were accompanied by nausea but no vomiting. In the second, death resulted in less than a minute from respiratory paralysis. The failure of these two experiments led us to try the administration of the required dose in two portions, as recorded in the protocol, with the result that emesis was readily produced showing the action to be central.

Nicotine and lobeline. These two alkaloids are so closely related in origin, composition and actions that they will be considered together. Both were used in the form of the sulphate. Two samples of nicotine were tested, one a commercial one, the other a specimen prepared by Professor Lloyd by precipitation with hydrous aluminum silicate. The activity of the two samples was found to be the same, so that the table is given as of nicotine

without particular reference to its source. The lobeline used was also supplied to us through the kindness of Professor Lloyd.

In the case of nicotine, the figures given in Table X show that the minimal emetic dose by mouth is about fifty times as great as that by vein, and the time required after oral administration is,

TABLE X
Nicotine sulphate

	MGM. PER KILO	EFFECT	TIME IN MINUTES	REMARKS
Vein.....	0.10	N	5	
	0.15	N	1	
	0.20	E	4½	
	0.30	E	11	
	0.50	N	2	Convulsive twitchings
	0.70	N	1	Convulsive twitchings
Oral*.....	9.00	E	56	
	10.00	E	45	
	10.00	E	8	
	10.00	N	9	
	10.00	E	3	

* Doses of 1.0, 2.0, 4.0, and 8.0 mg. per kilo were given without effect.

TABLE XI
Lobeline sulphate

	MGM. PER KILO	EFFECT	TIME IN MINUTES
Vein*.....	0.5	E	4½
	0.5	N	1
	0.6	N	1
	0.6	E	2½
	0.6	E	3
	0.7	E	3
Oral†.....	5.0	0	0
	6.0	0	0
	7.0	E	25
	8.0	E	9

* Doses of 0.3, 0.3, and 0.5 mgm. per kilo were given with the production of nausea only.

† Doses of 10.0, 20.0, 25.0 and 30.0 mgm. per kilo were given, all producing emesis in from 11 to 38 minutes.

in most cases, much longer than that after intravenous. Similar relations are seen to exist between the oral and intravenous doses of lobeline—Table XI—but the difference in this case is not so great. The oral dose is only about ten to fifteen times the intravenous, an indication of more complete or more rapid absorption, or of both. One other difference was noted between these two drugs, namely, that lobeline was found to be less active than nicotine when given by vein, although it seemed slightly more active when given orally. The latter difference is almost certainly due solely to its readier absorption.

Both drugs produced typical phenomena of emesis in eviscerated dogs, as the accompanying protocols show.

September 10, 1914. Male dog, 5.9 kilo.

12.20 p.m. Begin chloroform.

12.32. Chloroform withdrawn.

12.36. Operation completed; animal released.

1.10. Nicotine 1.0 mgm. per kilo intramuscularly

1.18. Nausea.

1.19:30. Emesis.

November 24, 1914. Female dog, 4.8 kilo.

9.50 a.m. Begin chloroform.

10.05. Chloroform withdrawn.

10.06. Operation completed; animal released.

11.59:15. Lobeline 0.7 mgm. per kilo into femoral vein.

12.00 p.m. Nausea.

12.04. Emesis.

Pilocarpine. The direct action upon the salivary glands is so marked that the symptom of nausea ceases to be a trustworthy one, as in both nausea and pure salivation the dog licks his chops frequently. Excluding, therefore, those doses which failed to produce emesis—Table XII—we again encounter a decided difference in the times required for the appearance of vomiting after intravenous and oral administrations, although the effective doses are not very far apart. The average time for emesis in the five intravenous administrations is 3 minutes, while that in the seven oral instances is almost 33 minutes, from which it is evident that absorption is slow.

TABLE XII
Pilocarpine nitrate

	MGM. PER KILO	EFFECT	TIME IN MINUTES
Vein*.....	0.60	N	1½
	0.60	N	1
	0.60	E	3½
	0.60	E	2
	0.75	N	2
	0.75	N	5
	0.75	N	1½
	0.75	E	2
	1.00	E	4
	1.00	E	3½
Oral†.....	0.75	N	23
	0.75	E	27
	1.00	N	30
	1.00	E	43
	1.50	N	27
	1.50	E	35

* Doses of 0.5 mgm. per kilo were given to six animals with the production of salivation and signs of nausea only.

† Doses of 1.5, 2.0, 2.5, and 3.0 mgm. per kilo were given, resulting in emesis in all cases in from 20 to 54 minutes.

The following protocol confirms this evidence of the central action of the drug in the production of vomiting:

September 3, 1914. Female dog, 6.0 kilo.

11.17 a.m. Begin chloroform.

11.30. Chloroform withdrawn.

11.33. Operation completed; animal released.

12.45 p.m. Pilocarpine 1.0 mgm. per kilo into femoral vein.

12.47. Salivation.

12.49. Nausea.

12.52. Emesis.

Physostigmine. Experiments were made with this drug, but it was not possible to establish any certainly emetic dose, although all the animals showed marked salivation and appeared to be nauseated. The failure to establish an emetic dose seemed to be due, in part, to an uncertainty in the production of this

action, and in part to the immediate production of very marked muscular incoördination and weakness with central depression which seemed to interfere with the vomiting act. One or two of the animals retched violently but did not vomit after intravenous administration, and only three vomited after intramuscular injection of the drug. Owing to this inability to fix a dose which would produce emesis with reasonable certainty we did not test the drug on eviscerated animals, but it would seem probable, in those cases in which vomiting follows its administration, that this is due to a central action. This, however, is based only upon the close similarity existing between the actions of physostigmine and pilocarpine on other portions of the nervous system and is not proved by direct experiment.

Aconitine and veratrine. The comparative unimportance of these two alkaloids in therapeutics, and their similar actions warrant their discussion together. Tables XIII and XIV give the results of the experiments undertaken to establish their minimal emetic doses and the times required for the production of emesis after both intravenous and oral administrations. The evidence derived from these figures is similar to that already discussed for several other drugs and need not be reviewed. The subjoined protocols complete the evidence proving the central action of both drugs in their production of emesis.

March 19, 1915. Female dog, 5.96 kilo.

2.17 p.m. Begin chloroform.

2.26. Chloroform withdrawn.

2.28. Operation ended: animal released.

3.06. Aconitine 0.035 mgm. per kilo into ear vein.

3.07. Nausea; incoördination.

3.07:20. Retching.

3.07:35. Emesis.

April 1, 1915. Male dog, 3.74 kilo.

10.31 a.m. Begin chloroform.

10.44. Chloroform withdrawn.

10.51. Operation completed: animal released.

11.08. Veratrine 0.2 mgm. per kilo into ear vein.

11.10. Emesis. Repeated at 11.12.

TABLE XIII
Aconitine hydrochloride

	MGM. PER KILO	EFFECT	TIME IN MINUTES
Vein.....	0.025	N	5
	0.025	E	11
	0.030	R	3½
	0.035	E	20½ Fatal
	0.050	E	4
Oral.....	0.035	O	0
	0.050	E	40
	0.075	N	30
	0.100	E	18
	0.200	E	28
	0.200	E	6

TABLE XIV
Veratrine sulphate

	MGM. PER KILO	EFFECT	TIME IN MINUTES	REMARKS
Vein*.....	0.050	O	0	
	0.050	E?	¼	Obvious attempted emesis at once
	0.050	E	1	
	0.075	E	½	Muscular incoördination
Oral.....	0.500	N	4	
	0.750	E	3	
	0.750	O	0	
	1.000	E	14½	
	1.000	E	4½	

* Doses of 0.03 mgm. per kilo to each of two dogs were without effect; doses of 0.1, 0.1, 0.2, 0.25, and 0.3 mgm. per kilo were also given, all producing emesis except 0.2 which caused retching only. All caused convulsions and muscular stiffness at once.

Attention is called to the extremely small doses of both of these alkaloids which suffice to produce emesis, and in one instance as little as 0.035 mgm. per kilo of aconitine intravenously killed a dog. The pronounced action of each of these drugs upon the muscles producing twitching, stiffness, and incoördination twice seemed to prevent emesis, retching alone being possible.

Ergot. Although vomiting is not often seen in man after the therapeutic use of ergot, it is stated to be a common symptom in some cases of poisoning by this drug. As the recently isolated alkaloid, ergotoxin, has not come into use as a substitute for ergot, and as the drug as ordinarily employed derives its actions from several principles, we thought it best to test it as a whole. The fluid extract was, therefore, used and was diluted with water or salt solution for administration Table XV. All doses of 60.0 mgm. per kilo (in terms of dry drug) or over, produced prompt emesis when given intravenously, whereas as much as a gram per kilo administered orally proved to be without this action. This alone shows a lack of local irritant action to account for the

TABLE XV
Ergot—fluid extract

	MGM. PER KILO	EFFECT	TIME IN MINUTES
Vein*.....	50.0	0	0
	60.0	E	6
	60.0	E	1 $\frac{1}{4}$
Oral.....	200.0	0	0
	400.0	N?	20
	800.0	N	8
	1000.0	0	0

* Doses of 50.0 mgm. per kilo to each of two dogs were without effect: doses of 75.0 and 100.0 mgm. per kilo each produced emesis in 8 $\frac{1}{2}$ and 5 $\frac{1}{2}$ minutes, respectively.

emesis, and the following protocol of an evisceration experiment serves to establish the action as a central one.

March 31, 1915... Female dog, 4.3 kilo.

10.45 a.m. Begin chloroform.

10.55. Chloroform withdrawn.

11.00. Operation completed: animal released.

11.40. Fl. ext. ergot 75.0 mgm. per kilo into ear vein.

11.42:30. Nausea.

11.44. Emesis.

Picrotoxin. The general actions of this drug suggest that it produces emesis by direct action on the center, and the experi-

mental results recorded in Table XVI and in the protocol of an evisceration experiment demonstrate the correctness of this assumption.

TABLE XVI

Picrotoxin

	MGM. PER KILO	EFFECT	TIME IN MINUTES
Vein*.....	0.2	0	0
	0.3	E	9
	0.4	E	3½
Oral†.....	2.0	N	10½
	2.5	E	10
	3.0	E	10

* Doses 0.1 and 0.15 mgm. per kilo were without effect; doses of 0.4 and 0.5 mgm. per kilo produced emesis and nausea, respectively.

† Doses of 1.0 and 2.0 mgm. per kilo were without effect, except for nausea after the second.

September 15, 1914. Female dog, 7.92 kilo.

2.12. p.m. Begin chloroform.

2.24. Chloroform withdrawn.

2.27. Operation completed: animal released.

2.54:30. Picrotoxin 0.4 mgm. per kilo into femoral vein.

2.55. Nausea.

2.55:15. Emesis

Sodium Salicylate. Nausea and vomiting are generally recognized as being among the commonest and most annoying of the side actions of this and of other salicylates. They are almost universally attributed to an irritant action upon the gastric mucosa and many suggestions have been made to prevent such action, such as the use of an excess of sodium bicarbonate or the employment of preparations which pass the stomach without decomposition. Recently, however, Hanzlik (12) suggested that the nausea and vomiting are "probably central." Conclusive evidence is also accumulating to the effect that those preparations which pass through the stomach without solution or decomposition are quite as likely to produce nausea and vomiting as are the soluble preparations such as the salicylate of sodium when they

are given in equivalent doses (in terms of the salicylic radical) (12) (13). It has already been shown by Waddell (14) that emesis in animals follows as frequently after intravenous administration of sodium salicylate as after oral doses, and the results of our experiments on dogs, recorded in Table XVII confirm this observation. Waddell was never able to find salicylates in the vomitus after subcutaneous administration. From Table XVII it is seen that the minimal emetic dose by oral administration is more than three times that for intravenous, and that the time before the onset of emesis after the former method is also usually greater than after the latter. An experiment on an eviscerated dog, the protocol of which is given below, completes the evidence in favor of the central emetic action of sodium salicylate.

TABLE XVII
Sodium salicylate

	MGM. PER KILO	EFFECT	TIME IN MINUTES
Vein*.....	200.0	N	1½
	200.0	E	4½
	300.0	E	2
	300.0	E	10
Oral†.....	500.0	0	0
	750.0	E	49
	750.0	E	8½

* Doses of 100.0, 150.0 and 150.0 mgm. per kilo were given, nausea alone following one dose of 150.0 mgm.

† Doses of 1000 mgm. each were given to two dogs with the production of emesis in both.

September 1, 1914. Female dog, 4.6 kilo.

10.08 a.m. Begin chloroform.

10.23. Chloroform withdrawn.

10.26. Operation completed: animal released.

10.55:30. Sodium salicylate 300 mgm. per kilo into femoral vein.

10.57. Emesis.

In two other evisceration experiments with this drug, one animal showed repeated attempts at emesis, and the other nausea but no actual attempt to vomit.

Atropine, cocaine and colchicine. We attempted to determine the emetic doses for each of these drugs upon dogs, but were unsuccessful through one cause or another. Apparently both cocaine and atropine are usually without such action in these animals, at least we failed to observe it with any dose which we employed up to those which were fatal. In the case of colchicine the action is very slowly elicited, owing to the fact that the drug must first undergo oxidation into oxydicolchicine in the body, and emesis was even then wanting after the doses which we used.

GENERAL DISCUSSION

Throughout this entire series of experiments it is to be observed that the smallest emetic oral dose was always larger than that required by intravenous administration, with but two exceptions, namely, the ipecac alkaloids, in which cases the doses for the two modes of administration were practically the same. In our previous studies on apomorphine and on the digitalis bodies the oral dose was also found to be materially larger than the intravenous dose. All of the drugs which showed this relation also required a longer interval for the production of emesis after their oral administration than when given intravenously, and this was also true of the ipecac alkaloids. It would seem, therefore, that the establishment of these two facts alone for any drug should be regarded as very strong evidence that its action is central. However, inasmuch as the evisceration experiment is comparatively simple, the conclusive evidence which it affords should be secured before a drug is finally regarded as being proved to act upon the vomiting center.

CONCLUSIONS

1. Practically all alkaloids and alkaloidal drugs in common use including morphine and preparations containing it; emetine and cephaeline quinine; nicotine and lobeline; pilocarpine; aconitine and veratrine; ergot and apomorphine, which produce nausea and vomiting, either as their chief or as their side actions, do so by direct action upon the vomiting center.

2. Sodium salicylate, picrotoxin and the digitalis bodies also produce nausea and emesis through direct central action.

3. There may be, in the case of certain drugs with irritant properties, synergistic reflex and central actions, whereby a smaller dose by mouth may be effective than would be the case for either action alone. An illustration of this seems to be afforded by emetine.

4. The evidence in favor of the central action does not in every instance exclude the possibility of the drug's also having an irritant action on the alimentary mucosa when given orally. It is probable that the ipecac alkaloids, veratrine and sodium salicylate may have this reflex action in man, at least in susceptible individuals.

5. Some of the advantages claimed for certain proprietary preparations of opium, such as, narcophin and pantopium are refuted by the results of the present experiments.

6. This is also probably true to a certain extent of some of the proprietary preparations of salicylic acid.

We wish to acknowledge the valuable assistance given us by Mr. Kaufman Wallach of the Second Year Class in Medicine in a considerable portion of this work, including both the determination of the minimal emetic doses and aid in the performance of some of the evisceration experiments.

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THE INFLUENCE OF SALICYLATES ON THE ELIMINATION OF URIC ACID AND OTHER WASTE PRODUCTS FROM THE BLOOD

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It has been known for more than thirty years that the administration of salicylic acid and its derivatives increases markedly the excretion of uric acid, and a large number of papers dealing with this phenomenon are to be found in both clinical and scientific journals.¹ The mechanism of this increased uric acid output is however not yet clear. The older observers believed it to be due to an increase in the total nitrogenous metabolism; Bohland² in 1896 advanced the idea that it resulted from the marked leucocytosis which has been described as following the administration of salicylic acid and its derivatives; while Hall³ and Rockwood⁴ ascribed it to a decrease in activity of the uricolytic enzymes of the tissues. During the past two years the writer has had occasion to examine a large number of specimens of blood from patients suffering from various forms of chronic and acute arthritis, and has been frequently astonished to obtain uric acid values as low as 0.5 mg. per hundred grams of blood.⁵ Examination of the clinical records invariably showed that patients

¹ For an extremely complete bibliography of the subject see Hanzlik, *The Salicylates: Annual report (1914) of the Therapeutic Research Committee of the Council on Pharmacy and Chemistry of the American Medical Association.*

² *Centralbl. f. inn. Med.*, 1896, 17, 70.

³ *British Medical Journ.*, 1904, 11, 744.

⁴ *Am. Journ. Physiol.*, 1909, 25, 34.

⁵ From results obtained on a large number of specimens Folin and Denis concluded that the normal amounts of uric acid in human blood is 1.5 to 2.5 mgm. per 100 grams of blood. *Journ. Biol. Chem.*, 1913, 14, 29.

whose blood gave abnormally low uric acid figures were receiving large doses of sodium salicylate or of acetyl salicylic acid (aspirin).

In view of these observations it has seemed worth while to make a few experiments under carefully controlled conditions concerning the action of salicylic acid and its derivatives on the blood.⁶

Such experimental data has a twofold value; on the one hand in throwing more light on the pharmacological action of the salicylate group of drugs, and on the other in furnishing a surer knowledge of the factors which must be taken into account when using the uric acid content of the blood as a diagnostic test for gout. All the subjects used in the following experiments were adult male patients in the medical wards of the Massachusetts General Hospital. With two exceptions they were kept in bed during the entire period of observation. A liberal but purin free diet was given during the experimental period and was in every case started one or two days before the collection of urine or blood was begun.

The analytical methods employed for the determination of uric acid in blood and urine and for the non-protein nitrogen of blood were those described by Folin and Denis,⁷ the total nitrogen of the urine was determined by the Folin-Farmer procedure.⁸

Two sets of experiments were carried out. In the first series (experiments 1-8) maximum doses of sodium salicylate or of acetyl salicylic acid were given, the drug being administered

⁶ After experimental work was underway the following note by Fine and Chace appeared in the Proceedings of the Society for Experimental Biology and Medicine, 12, p. 95.

"That the increased output of uric acid following the use of atophan is accompanied by a diminution in the concentration of uric acid in the blood appears to be well established. As has been long known, salicylates induce an increased elimination of uric acid quite comparable to that produced by atophan; and it is of interest to learn whether this urinary increase is accompanied by a commensurate decrease in the blood.

"We have found in seven cases that sodium salicylate in daily doses of 6 grams reduced the uric acid concentration of the blood to one-half to one-third of the initial concentrations."

⁷ Journ. Biol. Chem. (1913), 13, 469; 14, 95.

⁸ Journ. Biol. Chem., 1912, 11, 493.

every two hours during the day until unmistakable symptoms of intoxication were noted. This treatment was continued for three days.

In the second series (experiments 9-13) the administration of the drug was continued for one day only; the amount given being usually 3-7 grams.

The experimental results obtained are given below.

Experiment I. B. O. 31. 39 years old. Diagnosis: cardiac hypertrophy and mitral stenosis.

URINE				BLOOD (100 GRAMS)	
Day	Sod. salicylate	Nitrogen	Uric acid	Non-protein nitrogen	Uric acid
	grams	grams	grams	mgm.	mgm.
1.....	0	9.6	0.38	40	2.0
2.....	7	9.0	1.02		
3.....	7	8.5	0.51		
4.....	2.3	10.8	0.76	40	0.8
5.....	0	9.2	0.77		

Experiment II. C. A. 7. 49 years old, weight 163.6 K. In hospital for observation as to the cause of a mild indigestion of fifteen years duration.

URINE				BLOOD (100 GRAMS)	
Day	Asperin	Nitrogen	Uric acid	Non-protein nitrogen	Uric acid
	grams	grams	grams	mgm.	mgm.
1.....	0	10.2	0.40		
2.....	0	10.0	0.44	79	3.1
3.....	5.3	9.2	0.64		
4.....	8.0	10.5	0.70		
5.....	8.0	8.1	0.42	32	0.65
6.....	0	6.0	0.36		
7.....	0			37	1.0

Experiment III. D. O. 7. 39 years old, weight 72.7 K. Diagnosis: chronic infectious arthritis of unknown etiology.

URINE				BLOOD (100 GRAMS)	
Day	Sol. salicylate	Nitrogen	Uric acid	Non-protein nitrogen	Uric acid
	grams	grams	grams	mgm.	mgm.
1.....	0	11.6	0.42	28	10.0
2.....	0	11.0	0.48		
3.....	10.0	8.6	0.62		
4.....	11.0	9.3	0.64		
5.....	12.0	10.1	0.79	34	2.0
6.....	0	12.7	0.67		

Experiment IV. S. U. 7. 25 years old, weight 52.2 K. Diagnosis: Valvular disease both mitral and aortic.

URINE				BLOOD (100 GRAMS)	
Day	Sod. salicylate	Nitrogen	Uric acid	Non-protein nitrogen	Uric acid
	grams	grams	grams	mgm.	mgm.
1.....	0	11.5	0.41		
2.....	0	10.5	0.42	64	4.0
3.....	5.3				
4.....	8.0	13.8	0.50		
5.....	4.0	11.4	0.62	36	0.65
6.....	0	12.8	0.50	44	1.0

Experiment V. S. H. 31. 49 years old, weight 70 K. Cholelithiasis.

URINE				BLOOD (100 GRAMS)	
Day	Sod. salicylate	Nitrogen	Uric acid	Non-protein nitrogen	Uric acid
	grams	grams	grams	mgm.	mgm.
1.....	0	9.2	0.57	31	2.0
2.....	7	9.0	0.72		
3.....	2.3	6.1	0.43		
4.....	2.3	10.0	0.40	32	0.9
5.....	0	8.4	0.54		

Experiment VI. McC. 31. Negro, 43 years old, weight 60.4 K. In hospital for treatment of mild indigestion from which he has suffered for 14 years.

URINE				BLOOD (100 GRAMS)	
Day	Sod. salicylate	Nitrogen	Uric acid	Non-protein nitrogen	Uric acid
	grams	grams	grams	mgm.	mgm.
1.....	0	7.3	0.30	40	1.9
2.....	7	5.6	0.26		
3.....	7	5.2	0.27		
4.....	2.3	8.9	0.50	41	0.9
5.....	0	5.4	0.22		

Experiment VII. B. E. 31. 49 years old, weight 65 K. Diagnosis: coronary sclerosis with angina.

URINE				BLOOD (100 GRAMS)	
Day	Sod. salicylate	Nitrogen	Uric acid	Non-protein nitrogen	Uric acid
	grams	grams	grams	mgm.	mgm.
1.....	0	7.2	0.52	47	1.9
2.....	7	8.3	0.80		
3.....	5	7.7	0.53		
4.....	2	8.2	0.60	38	1.1
5.....	0	7.4	0.40		

Experiment VIII. O. C. 7. 48 years old, weight 84 K. Alcoholic. Sciatica. Gives history of typical attacks of gout. Tophi in ears.

URINE				BLOOD (100 GRAMS)	
Day	Sod. salicylate	Nitrogen	Uric acid	Non-protein nitrogen	Uric acid
	grams	grams	grams	mgm.	mgm.
1.....	0			40	4.1
2.....	8	14.5	1.50		
3.....	8	11.1	0.88		
4.....	8	10.8	0.82	32	0.4
5.....	0	9.1	0.73		
6.....	0	7.8	0.46		
7.....	0	12.0	0.90	32	2.0

Two grams aspirin given on 6th and also on 7th day.

Experiment IX. C. P. 31. 34 years old, weight 63.6 K. Chronic infectious arthritis with marked hypertrophic changes.

URINE				BLOOD (100 GRAMS)	
Day	Sod. salicylate	Nitrogen	Uric acid	Non-protein nitrogen	Uric acid
	grams	grams	grams	mgm.	mgm.
1.....	0			43	1.9
2.....	0				
3.....	0	10.8	0.59		
4.....	7	6.7	0.64		
5.....	0	5.4	0.48	32	2.0

Experiment X. M. O. 31. 36 years old, weight 60 K. Abdominal tumor.

URINE				BLOOD (100 GRAMS)	
Day	Sod. salicylate	Nitrogen	Uric acid	Non-protein nitrogen	Uric acid
	grams	grams	grams	mgm.	mgm.
1.....	0	9.9	0.56	27	1.8
2.....	*7	4.8	0.60		
3.....	0	7.2	0.52	28	0.6

* Sodium salicylate administered between 7.00 a.m. and 7.00 p.m. on day 2, blood drawn on day 3 at 7.30 a.m. The same procedure was adopted in Experiments XI, XII, and XIII.

Experiment XI. B. O. 31. 32 years old, weight 63.6 K. Slight endocarditis with possible mitral disease.

URINE				BLOOD (100 GRAMS)	
Day	Sod. salicylate	Nitrogen	Uric acid	Non-protein nitrogen	Uric acid
	grams	grams	grams	mgm.	mgm.
1.....	0	7.8	0.49	32	1.1
2.....	7	7.2	0.72		
3.....	0	7.3	0.25	38	0.6

Experiment XII. W. O. 7. 47 years old, weight 101 K. Cardiac decompensation, mitral insufficiency.

URINE				BLOOD (100 GRAMS)	
Day	Sod. salicylate	Nitrogen	Uric acid	Non-protein nitrogen	Uric acid
	grams	grams	grams	mgm.	mgm.
1.....	0	10.0	0.90	42	3.3
2.....	3.3	10.5	1.01		
3.....	0	7.2		28	3.3

Experiment XIII. I. A. 7. 30 years old, weight 109 K. Cardiac decompensation and fibrillation.

URINE				BLOOD (100 GRAMS)	
Day	Sod. salicylate	Nitrogen	Uric acid	Non-protein nitrogen	Uric acid
	grams	grams	grams	mgm.	mgm.
1.....	0	14.6	1.02	31	1.5
2.....	3.3	10.5	1.04		
3.....	0	7.9	0.99	32	1.6

As will be seen from the above results sodium salicylate and aspirin administered in the large doses frequently used in the treatment of acute rheumatic fever causes the uric acid content of the blood to be markedly reduced, a result which up to the present time has been shown for only one other drug, i.e., phenyl-quinolin carbonic acid (atophan).⁹ Most of the patients used in the work had blood giving non-protein nitrogen values but little above normal, in these the administration of salicylate had no effect on this fraction. In two or three cases, however, (Experiments 2, 4, 7, 8) it will be noted that the non-protein nitrogen is abnormally high and in these cases a considerable diminution of the fraction took place after the administration of the drug.

In the three cases in which large doses (7 grams) of sodium salicylate were given for one day only one showed no change in

⁹ Folin and Lyman: This Journal, 1913, 4, 539. Fine and Chace: Ibid., 1914, 6, 219.

the uric acid content of the blood while the other two showed a reduction comparable in size to that obtained in the first eight cases in which medication was continued for three days, a result similar to that obtained by Fine and Chace¹⁰ by means of atophan.

The smaller doses of sodium salicylate used in Experiments 12 and 13 were apparently without much effect in producing either an increased output of uric acid in the urine or a decreased content in the blood.

From the few cases in which I have been able to obtain a third sample of blood some days after the administration of salicylate had been stopped it would seem that as soon as the drug is withdrawn the accumulation of waste products recommences, a finding which is in accord with the long known fact that after the administration of salicylate is discontinued the output of uric acid in the urine is frequently diminished below the level at which it stood previous to giving the drug.

From the foregoing results the increased output of uric acid following salicylate medication is clearly due to a lowered threshold value of the kidney, not only for uric acid, but in all probability for other waste products as well. Such being the case, it may well be that the beneficial effects resulting from the use of salicylates in acute rheumatic fever, may in part at least, be due to a power possessed by this class of drugs of increasing kidney permeability, thereby facilitating the rapid and more or less complete excretion of the as yet unknown toxins which produce symptoms of these diseases.

¹⁰ This Journal, 1914, 6, 219.

STUDIES ON THE CIRCULATION IN MAN

XIV. THE CHANGE PRODUCED IN THE BLOODFLOW (IN THE HANDS) UNDER THE INFLUENCE OF DIGI- TALIS IN CASES OF AURICULAR FIBRILLATION.

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The marked beneficial action of digitalis in auricular fibrillation has been insisted upon by Mackenzie (Heart, ii, 265, 1911), Cushny (Heart, iv, 33, 1912), and other observers. We have thought it of interest to determine whether any effect can be shown to take place in the rate of the bloodflow through the hands, and have obtained a positive result in 3 cases, the flow being distinctly increased. The flow in patients under hospital regimen varies so little from day to day when the experimental conditions, especially the external temperature, are properly controlled, and no obvious clinical change has occurred, that it was not necessary to have recourse to intravenous injection of strophanthin. The administration of m20 of the tincture of digitalis every four hours *per os* was found sufficient to produce definite and prompt effects.

Thus in John Di C.,¹ a man aged 38 years, the flow immediately after admission to the hospital, before he had gone to bed, was 6.23 gm. per 100 cc. of part per minute for the right hand and 6.87 gm. for the left hand (average for the two hands 6.55 gm.) with an average room temperature of 24.8°C. Digitalis was begun about 24 hours after the bloodflow examination. Twenty hours after the first dose, the flow was found to be 8.56 gm. per 100 cc. of part per minute for the right and 8.28 gm. for the left hand

¹With one exception the cases studied were from the service of Dr. E. P. Carter, at the city hospital, to whom we are indebted for many courtesies.

(average for the two hands 8.42 gm.), with average room temperature 24.0°C. Twenty-four hours later, when he had received in all m220 of the tincture, the flows came out 10.80 gm. and 10.94 gm. for the right and left hands respectively (average 10.87 gm., with room temperature 25.0°C. His clinical condition had steadily improved, a distinct improvement being noted at the first examination after digitalis. At a fourth examination, eight days after admission, digitalis treatment being continued, the flows were 8.32 gm. and 8.26 gm. for the right and left hands respectively (average 8.29 gm.) with room temperature 26°C.

At the fifth examination, 11 days after admission, the flows were 8.02 gm. and 8.26 gm. for the right and left hands respectively (average 8.14 gm.) with room temperature 24.1°. These flows are still well above the initial level before digitalis was commenced, in spite of the fact that he complained of gastric discomfort. This was the last bloodflow examination in the case, and the digitalis was discontinued.

It may, of course, be supposed that some part of the improvement in the bloodflow is associated with the rest in bed and that the drug treatment is not necessarily responsible.

However, it is unlikely that the decided effect observed at the second and still more at the third examination could have been in any important degree due to rest alone. For the programme of making a second examination before digitalis was given had to be interrupted by the very circumstance that under rest alone the patient became so bad that digitalis had to be begun, 30 hours after his admission and before the control examination could be duplicated.

In the next case (Andrew S.) this objection was taken account of by keeping the patient under observation for two days in the hospital, making a bloodflow measurement on each day, and then beginning the administration of digitalis.

Andrew S., a man aged 65 years, had on the day of admission a flow of 6.79 gm. per 100 cc. of part per minute in the right hand and the same in the left hand, with room temperature 23.7°C. On the next day the flows were 6.96 gm. for the right and 6.70 gm. for the left hand (average 6.83 gm.) with room

temperature 24.2°C ., that is to say, exactly the same as on the day of admission. Clinically there was little if any change in his condition. Rest alone had not caused any change in the flow. It is proper to point out that such an exact correspondence on two successive days is merely accidental. But it can be safely concluded that any effect produced by rest was in this patient slight during these first 48 hours. Digitalis treatment was begun immediately after the second examination. On the following day (May 7), after he had received ml20 of the tincture, the flow in the right hand was 8.79 gm., and in the left 8.46 gm. (average 8.62 gm.) with average room temperature 24.7°C . On May 8, the flows were 7.53 gm. and 7.60 gm. for the right and left hands respectively (average 7.57 gm.) with room temperature 24.2°C . On May 10, when he had received altogether one ounce of the tincture, the flow in the right hand came out 10.17 gm. and in the left 9.87 gm. (average 10.02 gm.) with room temperature 24.9°C . His clinical condition was much improved.

On May 12, the flow in the right hand was 8.97 gm. and in the left 8.33 (average 8.65 gm.) with room temperature 26.2°C . The patient felt well except, as he said, that the medicine (digitalis) "made his heart beat too strong." This was the last examination of the case and digitalis was discontinued on this day.

In a third case (E. P. W.),² a prompt reaction to digitalis was also observed, but the patient developed pneumonia and only three examinations were possible. Three days after admission to the hospital and before digitalis had been administered, the bloodflow in the right hand was 6.9 gm. per 100 cc. per minute, and in the left 7.4 gm. (average 7.14 gm.) with room temperature 20.0°C . Digitalis was then begun and on the next day the flows came out 11.05 gm. for the right hand and 10.95 gm. for the left (average 11.0 gm.) with room temperature 19.8°C . It was observed that in E. P. W. the effect of the digitalis was very promptly manifested, the pulse rate declining very soon and

² This case was examined by one of us at the National Hospital for Diseases of the Heart, London, through the courtesy of Dr. Russell Wells.

diuresis becoming marked before the bloodflow measurement was made. Four days later, digitalis being continued, the flow in the right hand was 10.66 gm. and in the left 11.43 gm. per 100 cc. per minute (average 11.03 gm.) for the first 7 minutes in the calorimeters, with room temperature 17.5°C. Towards the end of the examination he began to feel chilly, the heating plant being out of order, and the bloodflow in the hands promptly diminished to 7.56 gm. per 100 cc. per minute for the right hand and 7.65 for the left (average 7.61 gm.) for the last 5 minutes in the calorimeters.

Putting the observations on these three cases together, the conclusion seems warranted that in auricular fibrillation when the heart responds in the typical way to digitalis the rate of the bloodflow in the peripheral vessels is increased. As has been pointed out elsewhere (*Archives of Int. Med.*, 1914, xiii, 1), an increased hand or foot flow, where no evidence of a purely local vasodilatation is present, must in general be interpreted as indicating an increased heart output.

It scarcely needs to be pointed out that if digitalis produces this effect, the hand flow need not be expected to go on increasing as long as the digitalis treatment is continued. On the contrary, and our observations lend support to this conclusion, it is to be assumed that a maximum effect will be reached which will be greater or less, and more or less promptly attained in different cases. Also it may be assumed that the continuance of digitalis beyond a certain point may diminish the flow instead of increasing it. Discontinuance of the digitalis will then be followed by an increased flow.

It is known also that in some cases in which auricular fibrillation is present, there is either no response to digitalis so far as the heart's action is concerned, or the response is much less striking than in the majority of cases. Our fourth case (C. R.), a syphilitic, seems to belong to this group. He had been treated with digitalis for a considerable time before the bloodflow observations were begun. The digitalis treatment had been combined with anti-syphilitic medication (mercury and salvarsan) and the latter was continued after digitalis was stopped. It was

not considered that he had responded well to digitalis. In any case after it was stopped his pulse did not become more irregular or more frequent, and he felt better at each examination. Although it is not desired to lay stress on this, it is of interest that there was no diminution in the hand flow after discontinuance of the digitalis treatment, but on the contrary a progressive, although moderate, increase. Thus at the first examination (May 19), after about 5 weeks' treatment, during which time the digitalis had been once discontinued for an interval of a few days, the flow in the right hand was 5.39 gm. per 100 cc. per minute, and in the left 4.56 gm. (average 4.98 gm.) with room temperature 24.5°C. Two days later digitalis was discontinued and on May 24 the flows were 6.59 gm. and 6.08 gm. for the right and left hands respectively (average 6.33 gm.) with room temperature 24.3°C. On May 27 the right hand had a flow of 7.12 gm. per 100 cc. per minute and the left hand a flow of 5.84 gm. (average 6.48 gm.) with room temperature 24.6°C. On June 1, when he was feeling so well that he wished to go home, the flows came out 7.78 gm. and 7.05 gm. for the right and left hands respectively (average 7.41 gm.) with room temperature 25.9°C. Of course the somewhat higher room temperature on this occasion might have been partly responsible for the increased flow, but it is unlikely that this was an important factor since the pulse rate was not increased.

The man left the hospital on June 3, and returned on June 8 feeling worse, with an increased pulse rate, dyspnoea on exertion, cough, and vomiting. The bloodflow in the hands three hours after his readmission was 6.68 gm. and 7.27 gm. for the right and left hands respectively (average 6.97 gm.), with room temperature 25.3°C. Digitalis was begun on the night of June 9 to 10, and the bloodflow again examined on June 11. He was feeling somewhat better, but he still had a good deal of cough, some dyspnoea, and his pulse, although the rate was somewhat diminished both at the apex and the wrist, was not markedly improved. The flow came out for the right hand 6.82 gm. and for the left 7.26 gm. (average 7.04 gm.) with room temperature 25.5°C., practically the same as at the last examination.

On June 12, digitalis having been continued in the meantime, a careful examination revealed no material improvement in the pulse, although the patient felt somewhat better.

On June 15, after he had received in all one ounce, three drachms of a tincture known to be active in other cases, the average hand flow was only 4.45 gm. per 100 cc. per minute with room temperature 25.0°C., i.e., about the same as at the end of the previous course of digitalis. The weather was rather cool and the room had to be heated artificially to some extent to obtain a room temperature comparable to that in the other observations. His hands felt distinctly cool. The pulse frequency at the wrist was only about half of the apex rate, showing that a very large proportion of the ventricular beats were too feeble to be detected in the radial. The radial pulse did not show the decided increase in volume, especially of the stronger beats under digitalis in the other three cases. No attempt has been made in these observations to separate a possible vasoconstrictor action of the drug on the bloodflow in the extremities from the effect of an increased heart output. If both effects are present in the three cases which exhibited a distinctly increased hand flow, it is to be assumed that the action on the heart more than offset vasoconstrictor action. It is conceivable that in different cases the relative magnitude of the two effects may be different. If both effects, for instance, were produced in C. R. vasoconstriction in the hands must have more than offset any increase in the output.

SUMMARY

In three cases with auricular fibrillation, the bloodflow in the hands was promptly and decidedly increased after the administration of digitalis. In a fourth case, which had not been considered to respond well to digitalis, the hand flow was somewhat increased when the drug was stopped after a rather long course of it. Digitalis having been again begun, the hand flow at the end of a week was again found to be diminished.

EXTRACTS FROM CASE HISTORIES

John Di C., an Italian laborer, aged 38 years, height 5 feet, 2½ inches, weight 131 pounds, admitted to the City Hospital May 24, 1915. Diagnosis: rheumatic myocarditis (and endocarditis) with auricular fibrillation. He had rheumatism at 12 years of age and two or three times since. He was in hospital nine years ago with heart trouble. He has had dizziness occasionally for the past two years, lasting only a minute or two at a time. His present illness began on Easter Sunday with pain in the chest. The feet were swollen for the following three or four days. He has not worked for the past six months on account of his illness. He cannot sleep because of pain and palpitation about the heart.

Heart. Left border of cardiac dullness at the anterior axillary line. No enlargement upward or to the right. Auscultation reveals a gross irregularity in the heart's action, some contractions being very weak with the sounds scarcely audible, others very forcible with loud and distinct sounds. Apex rate 135. Radial pulse unequal and irregular, with no predictable sequence. Blood pressure, average systolic 105.

The bloodflow in the hands was examined on May 24 immediately after his admission and before he had gone to bed. He had been resting in bed at home for a good many days before coming to the hospital.

May 25, Apex pulse rate 130, radial rate 110. Blood, leucocytes 15,000, haemoglobin 80 per cent, Wassermann test negative. Average systolic blood pressure 132. He got so bad on May 25 that it was necessary to start the administration of digitalis (mxx every four hours) at 11 p.m.

Urine: Trace of albumin with a few hyalin and granular casts.

The bloodflow was again examined on May 26. He said he felt better. Pulse 120 at the wrist.

May 27. Another bloodflow examination was made. He feels stronger today than at any time since he entered the hospital.

May 29. Apex rate 87, radial rate 85.

June 1. Pulse at wrist 60 and fairly regular. He feels fairly well. A bloodflow examination was made on this day.

June 4. The bloodflow was again examined today. He said he was feeling bad. He had just eaten his dinner, which he enjoyed, but he said "his stomach did not like it." Pulse at wrist 70. Figure 1 is an electrocardiogram from John Di C.

Andrew S., a Hungarian laborer, aged 65 years, admitted to the City Hospital on May 5, 1915. Diagnosis: chronic myocarditis with auricular fibrillation and arteriosclerosis. There is dyspnoea, and oedema of the legs below the knee. No cyanosis. Thorax emphysematous. Crepitant râles throughout, especially at the bases.

Heart: upper border of dulness third rib; left border one and one-half fingers' breadth outside the nipple line; right border inside the right sternal margin. Heart sounds faint. Systolic murmur at the apex. Radial pulse grossly irregular in rhythm and amplitude. Pulse rate at apex 150, radial 120.

The bloodflow was examined on day of admission. He is left-handed in his work, but in eating uses knife in the right hand. On May 6 another bloodflow examination was made. He has not had any drug

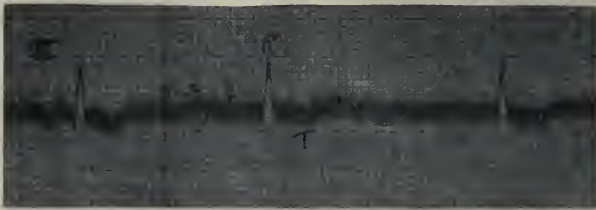


FIG. I. Case 1. Lead 2. Note the absence of the P summits and the marked irregularity of the ventricular deflection R.

treatment since admission. He still complains of dyspnoea. Blood, leucocytes 8200, haemoglobin 79 per cent. The highest radial pulse rate (120) was noted at 8 p.m. on this day. Digitalis (mxx of the tincture every 4 hours night and day) was begun at 4 p.m., on May 6, and continued till the evening of May 12. He had been in the hospital on a previous occasion and had then responded well to digitalis. Bloodflow examinations were made on May 7, 8, 10 and 12. On May 7 he said he felt better, though there was still some dyspnoea, and swelling of feet. He said he knew the "medicine" helped him. On May 8 his condition was much the same as on May 7. On May 10 further improvement was noted. Average systolic blood pressure 110.

On May 12 he said the medicine (digitalis) made his heart beat too strong. Otherwise he felt well. Still some swelling of the feet. The maximum radial pulse recorded on May 10-12 was 98.

E. P. W., a commercial traveller, aged 45 years, admitted to the hospital February 16, 1914. His illness began in October, 1909. Since then he has had gradually increasing symptoms—dyspnoea, palpitation, pain, cough and oedema—and he has been only able to work intermittently. His present breakdown dates from Christmas, 1913, since when his condition has rapidly grown worse.

Condition on admission. Dyspnoea, ascites, oedema marked in the lower extremities. Radial pulse 120–100, rhythm quite muscular. Arterial wall palpable. Jugular vein engorged. Limits of cardiac dulness, right 5 cm. from the midsternal line, left 15 cm. from midsternal line in fifth interspace. The electrocardiogram shows the characters of auricular fibrillation. There is some impairment of resonance at the bases of both lungs and many crepitations and râles. Liver enlarged, three inches below costal margin. The left arm has been weaker than the right and stiff since he was vaccinated in childhood. The first examination of the bloodflow in the hands was made on February 19. Digitalis was begun on the morning of February 20. He had been on digitalis some time before his admission to the hospital, and the effect of the present course was very promptly manifested, the pulse rate declining very soon and diuresis becoming marked before the second bloodflow examination was made on the afternoon of February 20. The digitalis was continued and a third bloodflow examination made on February 24.

C. R., a man aged 30 years, height 5 feet 10½ inches, weight 157 pounds, admitted to City Hospital April 15, 1915. He has worked as a collector and also at night as instructor in a gymnasium. He has had to give up his work on account of heart trouble. He had gonorrhea twice, 10 or 12 years ago, and chancre 6 or 7 years ago, when he underwent only local treatment for the sore. He has headache when he exercises too much, and dizziness at times, also dyspnoea and blurring of the sight. There is a slight oedema of the lower extremities below the knees. Blood, leucocytes 17,000; haemoglobin 85, Wassermann strongly positive.

Heart: left border 5 cm. outside mid-clavicular line; right border 2 cm. to right of right sternal margin. Upper border at third rib. A faint blowing systolic murmur is heard over the apex; otherwise the sounds are clear. The heart rate is grossly irregular and the sounds vary in intensity.

Diagnosis, syphilitic myocarditis with auricular fibrillation. He was put on digitalis and also on mercury (biniodide), and increasing doses of

potassium iodide. Apex rate 160, radial rate 110. Average systolic pressure 105. On May 2, the radial rate was 60, the apex rate 107. On June 1, the apex rate was 80 and the radial 72. Figure 2 is a polygraph tracing from C. R.

The bloodflow in the hands was measured on May 19. On May 21 digitalis was stopped, but the mercurial treatment was continued. On May 24 a second bloodflow examination was made. The patient said he felt better than at the last examination. A third examination was made on May 27. He was feeling very well and had been out in the

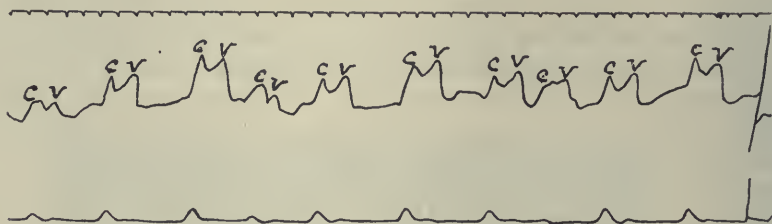


FIG. II. Tracing taken with Mackenzie polygraph from C. R. Upper curve from jugular, lower from radial artery. Time trace, fifths of a second.

yard. The maximum pulse rate at the wrist since digitalis was discontinued has been 84. Today it was 68. A fourth bloodflow examination was made on June 1. The pulse rate at the wrist is 72, at the apex 80. Since May 28 the pulse rate has varied from 84 to 60. He feels so well that he wants to go home. He went home on June 3, but returned to the hospital on June 8 complaining of dyspnoea and weakness, and that his stomach was upset. Apex rate 150, radial 115 on admission at 11 a.m. The bloodflow was examined on June 8, June 11 and June 15.

PROTOCOLS OF BLOODFLOW MEASUREMENTS

First examination of John Di C., May 24. Hands in bath at 5.03 p.m., in calorimeters at 5.17, out of calorimeters at 5.38. He was perspiring while his hands were in the calorimeters.

TIME	R	L	ROOM	TIME	R	L	ROOM
5.16	32.11	32.07		5.29	32.14	32.180	
5.18	32.09	32.07		5.30	32.15	32.190	24.6
5.19	32.09	32.07		5.31	32.15	32.195	
5.20	32.09	32.08	25.5	5.32	32.16	32.200	24.8
5.21	32.10	32.08		5.33	32.18	32.220	
5.22	32.10	32.08	25.2	5.34	32.20	32.230	24.8
5.24	32.11	32.10	25.1	5.35	32.22	32.250	24.6
5.25	32.11	32.11	24.7	5.36	32.23	32.280	
5.26	32.11	32.12		5.37	32.25	32.300	24.7
5.27	32.12	32.14	24.9	5.38	32.30	32.330	
5.28	32.14	32.16	24.8	5.53	32.16	32.180	

Cooling of calorimeters in 15 minutes, R 0.14°, L 0.15°C. Volume of right hand 344 cc., of left 332 cc. He is right handed. Water equivalent of calorimeters with contents, R 3370, L 3360. Rectal temperature 37.20°C.

Second examination of John Di C., May 26. Pulse 120 at wrist. Hands in bath at 3.31 p.m., in calorimeters at 3.43 $\frac{1}{2}$, out of calorimeters at 3.58.

TIME	R	L	ROOM	TIME	R	L	ROOM
3.43	31.36	31.32		3.52	31.600	31.560	
3.44	31.36	31.33		3.53	31.630	31.585	24.0
3.45	31.39	31.36	23.4	3.54	31.660	31.620	24.1
3.46	31.42	31.39		3.55	31.700	31.660	
3.47	31.44	31.41		3.56	31.730	31.680	24.1
3.48	31.46	31.43		3.57	31.755	31.700	
3.49	31.49	31.46	23.9	3.58	31.790	31.740	24.0
3.50	31.53	31.49		4.18	31.520	31.470	
3.51	31.57	31.52	23.9				

Cooling of calorimeters in 20 minutes 0.27°C. Volume of right hand 345 cc., of left 342 cc. Water equivalent of calorimeters with contents, R 3371, L 3368. Rectal temperature 37.67°C.

Third examination of John Di C., May 27. Pulse at wrist 92, another count 94, at apex 120. Hands in bath at 3.35 p.m., in calorimeters at 3.45 $\frac{1}{2}$, out of calorimeters at 4.00.

TIME	R	L	ROOM	TIME	R	L	ROOM
3.45	31.880	31.96		3.54	32.075	32.13	25.1
3.46	31.870	31.94		3.55	32.110	32.17	
3.47	31.880	31.95	24.8	3.56	32.140	32.20	
3.48	31.895	31.97		3.57	32.180	32.23	25.1
3.49	31.920	31.98		3.58	32.220	32.27	
3.50	31.945	32.00	25.0	3.59	32.250	32.30	
3.51	31.970	32.03		4.00	32.290	32.34	
3.52	32.010	32.07	25.0	4.06	32.220	32.27	
3.53	32.045	32.09					

Cooling of calorimeters in 6 minutes 0.07°C . Volume of right hand 352 cc., of left 342 cc. Rectal temperature 37.20°C . Water equivalent of calorimeters with contents, R 3376, L 3368.

Fourth examination of John Di C., June 1. The day was rather warm. Pulse at wrist 60, fairly regular. Hands in bath at $1.58\frac{1}{2}$ p.m., in calorimeters at $2.07\frac{1}{2}$, out of calorimeters at 2.28.

TIME	R	L	ROOM	TIME	R	L	ROOM
2.07	31.540	31.60		2.19	31.800	31.850	26.0
2.08	31.530	31.60		2.20	31.830	31.875	
2.09	31.555	31.62	26.1	2.21	31.860	31.890	
2.10	31.580	31.65		2.22	31.880	31.910	26.1
2.11	31.610	31.68		2.23	31.910	31.940	
2.12	31.640	31.70	26.1	2.24	31.940	31.970	26.1
2.13	31.670	31.72		2.25	31.970	32.000	
2.14	31.690	31.74		2.26	31.995	32.020	
2.15	31.700	31.76	26.1	2.27	32.015	32.050	26.1
2.17	31.740	31.79	26.0	2.28	32.050	32.080	
2.18	31.775	31.82		2.36	31.960	31.990	

Cooling of calorimeters in 8 minutes 0.09°C . Volume of right hand 351 cc., of left 342 cc. Water equivalent of calorimeters with contents, R 3376, L 3368. Pulse (at apex, with stethoscope) 60. Rectal temperature 37.35°C .

Fifth examination of John Di C., June 4. The day was rather cool. Pulse at wrist 70. Hands in bath at 12.27, in calorimeters at $12.36\frac{1}{2}$, out of calorimeters at 12.55.

TIME	R	L	ROOM	TIME	R	L	ROOM
12.36	32.030	32.080		12.47	32.20	32.285	
12.38	32.020	32.090	24.1	12.48	32.22	32.300	
12.39	32.040	32.110		12.49	32.24	32.320	24.10
12.40	32.060	32.130	24.2	12.50	32.25	32.340	
12.41	32.080	32.160		12.51	32.27	32.360	
12.42	32.100	32.180	24.1	12.52	32.29	32.375	
12.43	32.130	32.200		12.53	32.31	32.390	24.00
12.44	32.145	32.230		12.54	32.33	32.410	
12.45	32.160	32.260	24.2	12.55	32.35	32.430	24.05
12.46	32.190	32.275		1.04	32.23	32.310	

Cooling of calorimeters in 9 minutes 0.12°C. Volume of right hand 352 cc., of left 354 cc. Water equivalent of calorimeters with contents, R 3377, L 3379. Rectal temperature 37.02°C.

First examination of Andrew S., May 5. Hands in bath at 2.28 p.m., in calorimeters at 2.39½, out of calorimeters at 2.55.

TIME	R	L	ROOM	TIME	R	L	ROOM
2.39	32.090	32.120		2.48	32.14	32.20	24.0
2.40	32.060	32.090		2.49	32.17	32.22	
2.41	32.060	32.090		2.50	32.20	32.24	23.5
2.42	32.065	32.095	23.5	2.51	32.24	32.28	
2.43	32.070	32.100	23.2	2.52	32.26	32.31	23.6
2.44	32.065	32.110	23.1	2.53	32.29	32.33	
2.45	32.070	32.120	23.3	2.54	32.32	32.37	23.6
2.46	32.090	32.140		2.55	32.35	32.39	
2.47	32.110	32.170	23.8	3.45	31.77	31.77	

Cooling of calorimeters in 50 minutes, R 0.58°, L 0.62°C. Volume of right hand 461 cc., of left hand 465 cc. Water equivalent of calorimeters with contents, R 3464, L 3467. Rectal temperature 37.60°C.

Second examination of Andrew S., May 6. Hands in bath at 2.35 p.m., in calorimeters at 2.47, out of calorimeters at 3.05.

TIME	R	L	ROOM	TIME	R	L	ROOM
2.46	31.710	31.71		2.57	31.90	31.93	
2.48	31.710	31.71		2.58	31.94	31.970	
2.49	31.720	31.73	24.3	2.59	31.97	32.000	24.3
2.50	31.730	31.75	24.3	3.00	32.01	32.400	
2.51	31.740	31.77	24.2	3.01	32.05	32.060	24.1
2.52	31.750	31.79		3.02	32.07	32.080	
2.53	31.770	31.81	24.2	3.03	32.09	32.100	24.1
2.54	31.790	31.83		3.04	31.12	32.135	
2.55	31.830	31.87	24.3	3.05	32.14	32.160	
2.56	31.865	31.90		3.28	31.90	31.880	

Cooling of calorimeters in 23 minutes, R 0.24°, L 0.28°C. Volume of right hand 456 cc., of left hand 477 cc. Water equivalent of calorimeters with contents, R 3460, L 3477. Rectal temperature 37.59°C.

Third examination of Andrew S., May 7. Hands in bath at 3.40 p.m., in calorimeters at 3.50, out of calorimeters at 4.08. Pulse at wrist 96.

TIME	R	L	ROOM	TIME	R	L	ROOM
3.49	31.85	31.78		4.00	32.15	32.095	
3.51	31.85	31.78	24.7	4.01	32.18	32.120	24.5
3.52	31.87	31.80		4.02	32.23	32.180	
3.53	31.87	31.82		4.03	32.26	32.210	24.4
3.54	31.89	31.85	24.8	4.04	32.29	32.250	
3.55	31.93	31.89		4.05	32.34	32.290	24.5
3.56	31.98	31.93		4.06	32.37	32.320	
3.57	32.01	31.98	24.9	4.07	32.42	32.360	
3.58	32.07	32.01		4.08	32.46	32.400	
3.59	32.10	32.05	24.9	4.17	32.36	32.29	

Cooling of calorimeters in 9 minutes, R 0.10°C., L 0.11°C. Volume of right hand 467 cc., of left hand 480 cc. Water equivalent of calorimeters with contents, R 3469, L 3479. Rectal temperature 37.50°C.

Fourth examination of Andrew S., May 8. Weather colder than at last examination. Hands in bath at 11.20 a.m., in calorimeters at 11.30, out of calorimeters at 11.50.

TIME	R	L	ROOM	TIME	R	L	ROOM
11.29½	31.780	31.790		11.41	32.02	32.05	
11.31	31.790	31.795		11.42	32.05	32.08	24.1
11.32	31.795	31.805	24.1	11.43	32.08	32.11	
11.33	31.800	31.820		11.44	32.12	32.15	
11.34	31.810	31.850	24.0	11.45	32.15	32.19	24.1
11.35	31.840	31.880	24.0	11.46	32.18	32.21	
11.36	31.870	31.900		11.47	32.22	32.26	
11.37	31.900	31.930		11.48	32.26	32.29	24.1
11.38	31.930	31.960	24.3	11.49	32.29	32.32	
11.39	31.960	31.990		11.50	32.32	32.35	24.1
11.40	31.990	32.020	24.3	12.02	32.17	32.10	

Cooling of calorimeters in 12 minutes 0.15°C. Volume of right hand 471 cc., of left 463 cc. Water equivalent of calorimeters with contents, R 3472, L 3465. Pulse at apex 98. Rectal temperature 37.36°C.

Fifth examination of Andrew S., May 10. Hands in bath at 2.29 p.m., in calorimeters at 2.39½, out of calorimeters 2.55.

TIME	R	L	ROOM	TIME	R	L	ROOM
2.39	31.970	32.00		2.49	32.42	32.44	
2.41	32.020	32.05		2.50	32.47	32.49	
2.42	32.060	32.09		2.51	32.515	32.53	25.0
2.43	32.100	32.14	24.9	2.52	32.56	32.58	25.2
2.44	32.160	32.19		2.53	32.60	32.62	
2.45	32.200	32.23	24.8	2.54	32.65	32.67	25.1
2.46	32.265	32.29		2.55	32.70	32.70	
2.47	32.310	32.33	24.7	3.17	32.46	32.46	
2.48	32.370	32.39	24.9				

Cooling of calorimeters in 22 minutes 0.24°C. Volume of right hand 462 cc., of left 462 cc. Water equivalent of calorimeters with contents, R 3465, L 3465. Pulse 80 at wrist, 82 at apex. The strong pulse beats have a greater amplitude than at any of the previous examinations. Rectal temperature 37.75°C.

Sixth examination of Andrew S., May 12. Hands in bath at 2.50 p.m., in calorimeters at 3.02, out of calorimeters at 3.19. Pulse 76 (at wrist).

TIME	R	L	ROOM	TIME	R	L	ROOM
3.01	31.88	31.93		3.12	32.20	32.28	26.1
3.03	31.89	31.97	26.1	3.13	32.24	32.31	
3.04	31.92	31.99	26.5	3.14	32.29	32.35	26.2
3.05	31.94	32.01		3.15	32.33	32.38	
3.06	31.97	32.04	26.3	3.16	32.36	32.41	
3.07	31.99	32.08		3.17	32.29	32.44	26.3
3.08	32.04	32.12	26.2	3.18	32.43	32.48	26.3
3.09	32.08	32.17		3.19	32.48	32.51	26.1
3.10	32.12	32.20	26.2	3.30	32.36	32.49	
3.11	32.16	32.24					

Cooling of calorimeters in 11 minutes 0.12°C . Volume of right hand 452 cc., of left 457 cc. Water equivalent of calorimeters with contents, R 3456, L 3460. Rectal temperature 37.57°C .

First bloodflow examination of E. P. W., February 19. Hands in bath at 3.03 p.m., in calorimeters at 3.14, out of calorimeters at 3.30.

TIME	R	L	ROOM	TIME	R	L	ROOM
3.16	31.50	31.47		3.24	31.595	31.57	
3.17	31.52	31.48	19.2	3.25	31.60	31.585	20.0
3.18	31.525	31.49		3.26	31.61	31.60	
3.19	31.53	31.49		3.27	31.63	31.63	20.0
3.20	31.54	31.50	20.0	3.38	31.66	31.65	
3.21	31.56	31.52		3.29	31.67	31.66	
3.22	31.57	31.54	20.0	3.30	31.69	31.67	
3.23	31.58	31.56		3.42	31.45	31.44	

Cooling of calorimeters in 12 minutes, R 0.24° , L 0.23°C . Pulse 100. Volume of right hand 387 cc., of left 356 cc. Water equivalent of calorimeters with contents, R 3405, L 3380. Mouth temperature 36.35°C .

Second examination of E. P. W., February 20. Hands in bath at 3.44 p.m., in calorimeters at 3.55, out of calorimeters at 4.12.

TIME	R	L	ROOM	TIME	R	L	ROOM
3.57	31.19	31.36		4.06	31.51	31.655	19.9
3.58	31.22	31.42		4.07	31.56	31.670	
3.59	31.26	31.45	19.6	4.08	31.59	31.69	19.9
4.00	31.29	31.47		4.09	31.65	31.74	
4.01	31.33	31.50	19.7	4.10	31.68	31.76	20.0
4.02	31.36	31.53		4.11	31.72	31.79	
4.03	31.39	31.55	19.7	4.12	31.76	31.80	19.9
4.04	31.42	31.57		4.28	31.47	31.50	
4.05	31.495*	31.64*					

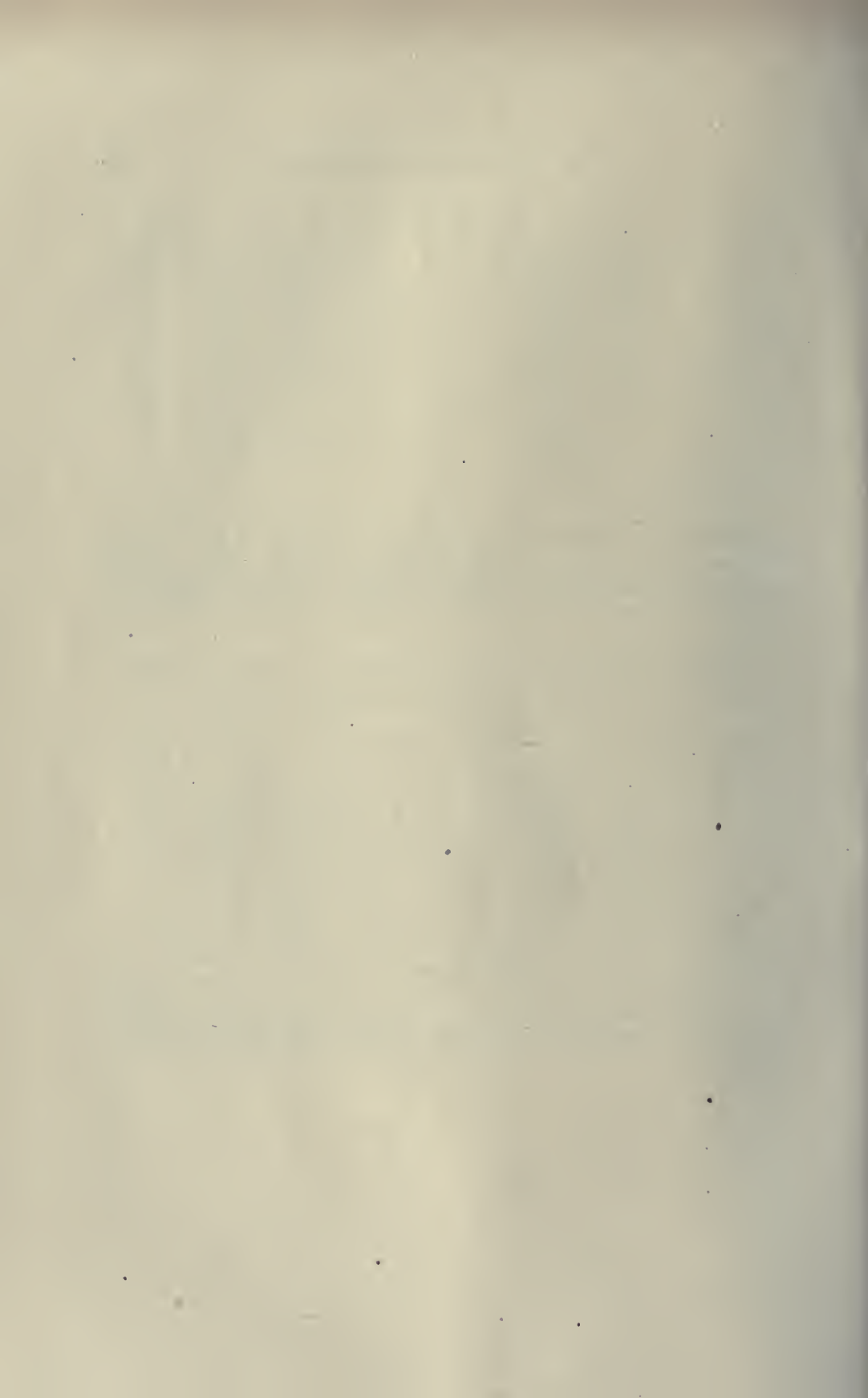
* Reading verified.

Cooling of calorimeters in 16 minutes, R 0.29°, L 0.30°C. Pulse 92 (amplitude considerably greater than at first examination). Mouth temperature 36.35°, rectal temperature 36.63°C. Volume of right hand 394 cc., of left 344 cc. Water equivalent of calorimeters with contents, R 3410, L 3370.

Third examination of E. P. W., February 24. Hands in bath at 3.00 p.m., in calorimeters at 3.11, out of calorimeters at 3.25.

TIME	R	L	ROOM	TIME	R	L	ROOM
3.13	32.09	32.29		3.20	32.26	32.44	
3.14	32.11	32.32	17.3	3.21	32.27	32.45	17.5
3.15	32.13	32.34		3.22	32.28	32.46	
3.16	32.16	32.36	17.4	3.23	32.30	32.465	17.5
3.17	32.17	32.37	17.5	3.34	32.31	32.47	
3.18	32.20	32.39		3.25	32.31	32.47	
3.19	32.23	32.42		3.39	32.00	32.14	

Cooling of calorimeters in 14 minutes, R 0.31°, L 0.33°C. Pulse 62. Rectal temperature 37.13°C. Volume of right hand 369 cc., of left 346 cc. Water equivalent of calorimeters with contents, R 3390, L 3372.



STUDIES ON THE CIRCULATION IN MAN

XV. FURTHER OBSERVATIONS, CHIEFLY PHARMACOLOGICAL, ON THE CRITERIA BY WHICH DEFICIENCIES IN THE BLOODFLOW (IN THE HANDS OR FEET) DUE TO MECHANICAL CAUSES MAY BE DISCRIMINATED FROM CHANGES DUE TO FUNCTIONAL (VASOMOTOR) CAUSES

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In Paper XIII of this series¹ I have investigated certain criteria by which a deficiency in the bloodflow through the hands or feet, or through one hand or foot, due to mechanical causes (embolism, compression or ligation of arteries) is distinguished from a deficiency due to vasoconstriction. One of these criteria is the constancy of the ratio of the flow in a mechanically obstructed part to the flow in a normal part in the same individual in successive measurements made at not too long intervals and under approximately the same external conditions, especially the same external temperature. Another is the relatively small response of the flow in the mechanically obstructed part to conditions which cause general cutaneous vasodilatation, especially a considerable increase in the external temperature. A third criterion investigated is the relatively feeble vasomotor reflex in the mechanically obstructed part when the contralateral part is immersed in warm or cold water. None of these criteria holds good for a deficient flow due to a functional (vasomotor) cause. The ratio of the flow in a part which happens at one time to have a deficient

¹ Journal of Experimental Medicine, 1915, xxii, No. 1.

circulation owing to increased vasoconstriction to the flow in a part in the same person with normal circulation does not remain constant from day to day. The ratio is easily altered when the external temperature is changed, and the flow in the affected part is readily influenced reflexly from the contralateral part. In this paper I desire to supplement these observations, in particular by observations in which the stability of the flow in the part in which it was deficient was tested under the administration of drugs causing vasodilatation (nitroglycerine, alcohol). It is clear that such drugs might be expected to affect the flow in a part with mechanically obstructed circulation to a smaller extent than the flow in a part whose circulation was diminished by vasoconstriction. The conclusion was verified in a number of cases.

John G.,² a Polish laborer, aged 41 years, came under observation for examination of the bloodflow at Lakeside Hospital on March 25, 1915. He had had his left leg amputated in the middle of the lower leg in July, 1909, and his right leg amputated at the middle of the thigh in May, 1913, for thrombo-angitis obliterans. He complains of pain in the hands, especially the left. The terminal phalanx of the left middle finger shows a gangrenous area at the tip and around the foot of the nail, which has existed for six weeks. There is discoloration, but the skin is unbroken as yet. There is no history of injury. The brachial pulse is well felt in the left arm, but the radial not. The patient says no pulse has been felt at his left wrist since it was fractured a while ago. The right radial pulse is good.

The bloodflow on March 25, 1915, was 10.32 gm. per 100 cc. per minute in the right hand and 8.6 gm. in the left, with room temperature 22.0°C. before the testing of the vasomotor reaction. During immersion of the right hand in cold water the flow in the left was only diminished to 7.90 gm. per 100 cc. per minute for the whole period of immersion of 9 minutes. During a 10 minutes immersion of the right hand in warm water the flow in the left was increased only to 8.08 gm. per 100 cc. per minute. That is to say, there were practically no vasomotor reflexes in the left hand from the contralateral hand, indicating a high degree of immobility of the vessel walls.

On April 27, 1915, the bloodflow in the hands was again examined

² I am indebted to my colleague, Dr. G. W. Crile, for the opportunity of studying this case.

with a very much higher room temperature. The gangrenous region on the terminal phalanx of the left middle finger had increased in area and depth. There was some pain in the hand from time to time but no pain in the necrosed area. The points of the little and ring fingers of the left hand, as well as the point of the middle finger were painful now.

The flow in the right hand was 11.29 gm. per 100 cc. per minute for 9 minutes before the vasomotor reflexes were tested, in the left only 5.64 gm., with room temperature 28.5°C. The diminution in the blood in the left hand as compared with the first examination, notwithstanding the high external temperature, is definite proof of the marked deterioration of the circulation in that hand in the interval. The great deficiency in the left hand as compared with the right in the face of the high temperature is conclusive evidence of mechanical obstruction. The vasomotor reflexes from the contralateral hand were again practically absent, or at any rate without influence on the bloodflow.

On April 30 the influence of nitroglycerine on the hand flow was tested. The day was cold, but it is quite characteristic of such conditions that the flow in the left hand was almost as great as on April 27 when the weather was warm (5.31 gm. per 100 cc. per minute for 7 minutes before the administration of nitroglycerine); whereas the flow in the right hand was diminished to 8.28 gm. per 100 cc. per minute (ratio of flow in left hand to that in right 1:1.559) with room temperature 23.4°C. For the first 4 minutes after the administration of nitroglycerine had begun, the flow in the right hand was 8.45 gm. per 100 cc. per minute as compared with 5.74 gm. in the left hand (ratio 1:1.472). For the next 12 minutes with further administration of nitroglycerine the flow in the left hand remained at 5.8 gm., while that in the right increased to 9.36 gm. per 100 cc. per minute (ratio 1:1.613). The change in the ratio shows that the vasodilatation has affected the right hand more than the left. Nausea now came on. The patient felt sick and his face became markedly pale for 3 minutes. During this period the flow in the right hand fell to 4.46 gm. and that in the left hand to 4.07 gm. per 100 cc. per minute (ratio 1:1.09). The great change in the ratio shows clearly that the diminution in the hand flows was not due mainly to a central cause (inhibition of the heart), or to a vasomotor effect produced elsewhere than in the extremities (e.g., in the splanchnic area), but to a peripheral vasoconstriction naturally affecting the right hand with its relatively mobile vessels far more than the left hand.³

³ Stewart: *Jour. Pharmacol. and Exper. Therap.*, 1911, ii, 481.

With the recovery from nausea the pallor of the face disappeared, and the flow in the right hand increased far more than that in the left (to 8.26 gm. per 100 cc. per minute for the right hand as compared with 4.32 gm. for the left for the last 10 minutes of the experiment, ratio 1:1.91).

The great relative increase in the flow in the right hand in the last 10 minutes of the experiment as compared with the left hand indicates the passing off of the vasoconstriction associated with the period of nausea. The left hand owing to the anatomical changes in the vessels is naturally but little affected. The flow in the left hand does not return to its initial value either because the driving power of the heart is still reduced or because a general vasodilatation still keeps the blood pressure lower than at first. If the blood pressure is lowered and the mechanical conditions do not permit appreciable diminution of the total vascular resistance in the left hand, the flow in that hand must be diminished, while the flow in the normal or approximately normal right hand may even be increased.

The effect of alcohol (or rather of certain alcoholic beverages) on the hand flow was first studied in a normal man (M. C.), 26 years of age. He had on many previous occasions been used as a subject, so that the limits of range of his hand flow were known.

For 10 minutes before the administration of alcohol, the flow was 14.74 gm. per 100 cc. per minute for the right hand and 13.58 gm. for the left (ratio 1:1.08) with room temperature 23.2°C. The first effect of alcohol (in the form of port wine) was to diminish the flow in both hands. This initial diminution has also been seen in the other cases. The flow for the first 10 minutes after the administration of the wine was begun was 12.81 gm. per 100 cc. per minute for the right hand and 11.18 gm. for the left hand (ratio 1:1.14). The diminution was not the same in the two hands, being proportionally greater in the left, and therefore it could not have been due solely to an action on the heart or to a vasomotor effect elsewhere than in the extremities. It must have been due partly at least to a vasoconstriction in the hands, and there is no obvious reason to expect that such a vasoconstriction should be exactly the same on the two sides. The initial effect of the alcohol is very promptly

manifested, certainly within the first minute. This is true of whisky as well as wine. It would therefore seem probable that it depends upon a vasomotor reflex liberated from the mucous membrane of the mouth, oesophagus or stomach. The same result was seen in another man in normal health but exhibiting a peculiarity in the hand flow which will be mentioned in the proper place. Neither of the subjects was an habitual drinker. The wine was relished by both, but the whisky, diluted with an equal volume of water, was not so well liked. In a patient with cardiorenal disease whisky also caused a diminution in the hand flow for the first 10 minutes after its administration. The whisky was not well liked in this case either. This is mentioned because the handflow is very easily influenced by psychical events, disgust, fear or painful impressions causing a prompt and decided diminution. But the fact that the wine caused a similar effect, although it was relished, makes it probable that in the case of the whisky also it is the reflex vasomotor effect and not a psychical reaction which is responsible for the initial vasoconstriction.

The next effect of the alcoholic beverage on the hand flow is an increased circulation in both hands. In M. C. for the second 10 minutes of the alcohol period the flow in the right hand was 16.77 gm. per 100 cc. per minute and in the left hand 15.76 gm. (ratio 1:1.06). For the next 7 minutes of the alcohol period, the flows were still further increased to 18.32 gm. and 17.61 gm. per 100 cc. per minute for the right and left hand respectively (ratio 1:1.04). The decline in the ratio shows that the initial moderate difference in flow in the two hands becomes continuously less as the absolute value of the flows increases. This could not be due to central changes alone or to vasomotor changes in other regions affecting both hands indirectly and therefore equally. It follows that some portion of the increased flow in the hands must be due to vasodilatation in the hands themselves. It is obvious that if the initial vasoconstriction was somewhat greater in the left hand than in the right, as vasodilatation under the influence of the alcohol increased, this difference would tend to disappear.

An interesting point well brought out in this experiment, and also observed in others, is that alcohol favors reflex vasodilatation in the hands. Thus, 27 minutes after the administration of alcohol was commenced the left hand was immersed in warm water. The initial diminution of the flow in the right hand, which is normally seen, was very slight and transient (from 18.32 gm. to 17.81 gm. per 100 cc. per minute for the first 3 minutes of immersion of the left hand in the warm water). Then followed a marked increase in the flow, which despite its previous high level rose to 23.57 gm., much the largest flow seen in M. C. on the twenty or more occasions on which his hand flow was measured in the past four years. This observation suggests that one way in which alcohol produces dilatation of cutaneous vessels is by so altering the response of the vasomotor centres to reflex stimuli that vasodilatation is favored. How far "paralysis" of vasoconstrictor tone by a direct depressant action on the vasomotor centres is a factor is not indicated by our observations.

The other normal, or at least healthy man (John R., 22 years old), in whom the effect of alcohol on the hand flow was investigated presents the peculiarity, not hitherto observed in any other healthy person, that the flow in the left hand is permanently very decidedly smaller than in the right. This has been the case in tests made over a period of more than two years. The ratio of the flows in the two hands remains so stable as to suggest a mechanical cause for the difference, for example a congenital difference in cross section of the two subelavians. The suggestion that a mechanical and not a vasomotor factor underlies the difference in flow is strengthened by the fact that conditions which cause considerable variations in the absolute amount of the hand flow do not tend to equalize the flow in the two hands.⁴ This idea is supported by the alcohol observations. For the 10 minutes before the administration of strong port wine, the flow in the right hand was 18.52 gm. per 100 cc. per minute, and that in the left hand 14.01 gm. (ratio 1:1.32), with room temperature 25.0°C.

⁴ Journal of Experimental Medicine, 1915, xxii, No. 1.

For the first two minutes after the taking of wine was begun the flow in both hands was diminished, the diminution being proportionally much greater in the right hand than in the left, so that the flow became about equal in the two hands.⁵ The fact that the diminution was so much greater in the right hand shows that it must have been due at least partly to vasoconstriction affecting the hands, although a temporary decrease in the output of the heart is not excluded. Just as in the case of John G. during the period of nausea, the vasoconstriction would necessarily tend to equalize the flow in the two hands if the flow in one were already diminished by a mechanical cause. Of course the same would be true if the vasoconstrictor tone of one hand was already greater than that of the other at the time the fresh vasoconstriction occurred. But we know that this is not the explanation in the case of John G., and that it is not the explanation in the case of John R. is indicated by the fact that for the next 8 minutes of the alcohol period when vasodilatation was already marked, a decided inequality in the two hand flows had already returned, the flow in the right being 19.56 gm. per 100 cc. per minute and that in the left 15.87 (ratio 1:1.23). For the next 10 minutes the flows were 20.70 and 16.14 gm. per 100 cc. per minute for the right and left hands respectively (ratio 1:1.28) and for the remaining 14 minutes of the experiment 21.99 gm. per 100 cc. per minute for the right hand against 17.07 gm. for the left (ratio 1:1.29, approximately the same as before the administration of alcohol.) These flows are also absolutely the largest ever observed in this individual.

The initial diminution in the hand flow after alcohol was also seen in Otis S., a man suffering from cardiorenal disease (chronic interstitial nephritis and myocarditis), with liquid in the right pleural cavity and the abdominal cavity and oedema of the arms, hands, legs and feet. About an hour after the aspiration of 2 litres of liquid from the right thorax, an examination of the flow

⁵ It must be pointed out that too much stress must not be laid on the apparent equality of the flows here because the error in calculating the flow for so short a period as two minutes would be 10 per cent if an error of 0.01°C. were made in a thermometer reading.

in the hands was made, during the course of which the patient was given 2 ounces of whisky followed by some water. He said he did not like the whisky and its administration was followed by flatulence. The observations were only continued for 10 minutes after the whisky was given. The flow in the right hand for the 9 minutes preceding the giving of alcohol was 6.83 (7.98)⁶ per 100 cc. per minute, and that in the left hand 7.94 (9.11) gm. (ratio 1:1.14) with the very high room temperature 29.8°C. For the 10 minutes after the alcohol was given the flows were 5.93 (6.92) gm. and 6.40 (7.34) gm. per 100 cc. per minute for the right and left hands respectively (ratio 1:1.06). For the first minute of the alcohol period the flows were much more decidedly diminished, to 4.2 (4.9) gm. for the right hand and 4.1 (4.6) gm. for the left.

SUMMARY

1. To the criteria already described which can be employed to discriminate between deficiency in the bloodflow (in the hands or feet) due to mechanical causes and deficiency due to vasomotor action, may be added the behavior of the flow when drugs which cause vasodilatation (nitroglycerine, alcohol) are administered.

2. Alcoholic beverages (wine, whisky) cause first a diminution and then an increase in the hand flow.

PROTOCOLS

First bloodflow examination of John G., March 25, 1915. Hands in bath at 2.42 p.m., in calorimeters at 2.52½. At 3.05 right hand put into water at 8.2°C.; at 3.14 into water at 43.7°C. At 3.24 left hand removed from calorimeter.

⁶ The numbers in parentheses are the flows calculated on the true volume of the hand tissue after deducting the oedema fluid. The true volume was obtained by measurements made when the hands were free from oedema.

TIME	TEMP. OF CALORIMS		ROOM	TIME	TEMP. OF LEFT CALORIM	ROOM	TIME	TEMP. OF LEFT CALORIM	ROOM
	Right	Left							
2.52	32.220	32.190		3.06	32.66	22.1	3.19	33.04	22.2
2.54	32.270	32.240	21.8	3.07	32.69		3.20	33.06	
2.55	32.310	32.270	22.0	3.08	32.74	22.0	3.21	33.09	22.15
2.56	32.360	32.320	21.9	3.09	32.755	21.9	3.22	33.12	22.2
2.57	32.400	32.360		3.10	32.785		3.23	33.15	22.2
2.58	32.450	32.400	21.9	3.11	32.81	22.0	3.24	33.17	
2.59	32.500	32.450	21.9	3.12	32.84				
3.00	32.550	32.480	21.9	3.13	32.87	22.0	3.37	32.96	
3.01	32.590	32.520	22.0	3.14	32.905		3.37	(Rt. 32.23)	
3.02	32.630	32.550	22.0	3.15	32.94	22.15			
3.03	32.680	32.580	22.0	3.16	32.96				
3.04	32.720	32.610	22.0	3.17	32.98	22.2			
3.05	32.770	32.645		3.18	33.01	22.2			

Cooling of calorimeters, right 0.54° in 32 minutes, left 0.21° in 13 minutes. Pulse 84. Volume of right hand 522 cc., of left 513 cc. Rectal temperature 37.53° . Water equivalent of calorimeters with contents, R 3512, L 3505.

Second bloodflow examination of John G., April 27, 1915. Hands in bath at 2.09 p.m., in calorimeters at 2.21. At 2.32 right put into water at $9.5^{\circ}\text{C}.$, and at 2.39 into water at $44.1^{\circ}\text{C}.$ At 2.47 left hand removed from calorimeter.

TIME	R	L	ROOM	TIME	R	L	ROOM
2.20.....	32.00	31.95		2.36		32.410	
2.22.....	32.07	32.00	28.4	2.37		32.440	28.4
2.23.....	32.12	32.03		2.38		32.460	
2.24.....	32.20	32.06	28.5	2.39		32.485	
2.25.....	32.27	32.10		2.40		32.510	28.6
2.26.....	32.33	32.13	28.5	2.41		32.530	
2.27.....	32.39	32.16		2.42		32.550	28.8
2.28.....	32.45	32.19	28.7	2.43		32.575	
2.29.....	32.52	32.22		2.44		32.600	
2.30.....	32.58	32.25	28.7	2.45		32.625	29.0
2.31.....	32.62	32.27		2.46		32.650	
2.32.....	32.69	32.29		2.47		32.670	
2.33.....		32.32	28.5	2.48	32.56		
2.34.....		32.35		3.01	32.46	32.560	
2.35.....		32.38	28.5				

Cooling of calorimeters R, 0.10° in 13 minutes, L 0.11° in 14 minutes. Volume of right hand 549 cc., of left 527 cc. Water equivalent of calorimeters with contents R 3534, L 3516. Rectal temperature 37.45°C . Blood pressure, right arm 185, 100 (sound gone).

Third bloodflow examination of John G., April 30, 1915. From time to time during the examination nitroglycerine (Spiritus Glonoini) was administered on the tongue. Hands in bath at $2.10\frac{1}{2}$ p.m., in calorimeters at $2.20\frac{1}{2}$, out of calorimeters at 2.58. Pulse 96.

TIME	R	L	ROOM	NOTES	TIME	R	L	ROOM	NOTES
2.20	31.79	31.840			2.40	32.58	32.27		
2.21	31.79	31.830			2.41	32.63	32.29	23.7	
2.22	31.85	31.860	23.2		2.42	32.67	32.31		
2.23	31.89	31.885	23.3		2.43	32.71	32.33	23.7	
2.24	31.95	31.910			2.44	32.74	32.35	23.7	Face getting pale.
2.25	31.995	31.930	23.4		2.45	32.79	32.36		Face pale. Yawns. Nausea.
2.26	32.035	31.950			2.46	32.80	32.37		
2.27	32.080	31.970	23.5		2.47	32.81	32.38		Feels sick, but does not want to vomit.
2.28	32.115	31.990	23.5	Pulse 102	2.48	32.82	32.39		Pulse 85. Volume of carotid pulse much reduced.
2.29	32.14	32.010		2 drops Sp. Gl.	2.49	32.86	32.41		He says he is now all right. Tongue and lips pale.
2.30	32.18	32.030	23.5		2.50	32.88	32.42		
2.31	32.22	32.050	23.5		2.51	32.90	32.43	23.8	Yawns. Some noise in ears.
2.32	32.270	32.080		Pulse not increased	2.52	32.93	32.44		Pulse 80. Sweats on face.
2.33	32.30	32.100	23.5	3 drops Sp. Gl.	2.53	32.97	32.45		Not so pale now.
2.34	32.35	32.130	23.5	No flushing of face	2.54	33.00	32.46	23.7	Pulse 88; volume much better.
2.35	32.395	32.150		Head a little sore but no throbbing	2.55	33.03	32.475		
2.36	32.42	32.180	23.6		2.56	33.07	32.48		Feels back cold.
2.37	32.46	32.200	23.6		2.57	33.09	32.49	23.9	
2.38	32.50	32.230		5 drops Sp. Gl.	2.58	33.11	32.50		
2.39	32.54	32.250	23.7		3.07	32.97	32.37		Pulse 89

Cooling of calorimeters, R 0.14° , L 0.13° in 9 minutes. Volume of right hand 544 cc., of left 521 cc. Rectal temperature 37.35°C . Water equivalent of calorimeters with contents, R 3530, L 3512.

Examination of bloodflow in M. C. to test influence of alcohol. Hands in bath at 3.18 p.m., in calorimeters at 3.28. Pulse at beginning of observations 94. At 3.40, 70 cc. port wine given; at 3.41, 70 cc. more; at 3.44, an additional 70 cc. At $3.49\frac{1}{2}$ p.m., he got 25 cc. whisky diluted with an equal volume of water. "It takes his breath."

TIME	R	L	ROOM	TIME	R	L	ROOM	NOTES
3.27	31.90	31.88		3.53	33.320	33.080	23.3	
3.29	31.98	31.86	23.2	3.54	33.380	33.150		Head heavy, feels sleepy and tired.
3.30	32.06	31.95		3.55	33.430	33.190		Pulse 88.
3.31	32.11	32.04		3.57	33.530	33.300		
3.32	32.19	32.09		2.58	33.590	33.350		
3.33	32.27	32.15		3.59	33.630	33.400		
3.34	32.33	32.21	23.2	4.00	33.690	33.450	23.3	
3.35	32.40	32.26		4.01	33.740	33.510		
3.36	32.47	32.32		4.02	33.790	33.555	23.5	
3.37	32.53	32.38	23.2	4.03	33.840	33.600		Head dizzy.
3.38	32.61	32.45		4.04	33.890	33.650		
3.39	32.68	32.50	23.2	4.05	33.940	33.750		
3.40	32.72	32.55		4.06	33.980	33.750		
3.41	32.77	32.57		4.07	34.020	33.790		Left hand put in water at 43.5° C.
3.42	32.79	32.60		4.08	34.060		23.4	
3.43	32.84	32.64		4.09	34.095			
3.44	32.89	32.68		4.10	34.140			
3.45	32.91	32.70		4.11	34.190		23.3	
3.46	32.96	32.75	23.2	4.12	34.240			
3.47	33.00	32.78		4.13	34.290		23.2	Feels sleepy and warm.
3.48	33.07	32.84		4.14	34.340			
3.49	33.11	32.88		4.15	34.390			
3.50*	33.17	32.94	23.3	4.16	34.430			Feels effect of alcohol decidedly.
3.51	33.21	32.97		4.17	34.490		23.3	Hand removed from calorimeter.
3.52	33.27	33.04		4.25	34.350	33.50		

* At this point he says he feels warm all over. Before this he only felt warm "inside."

Cooling of calorimeters, R 0.14°C. in 8 minutes, L 0.29°C. in 18 minutes. Volume of right hand 512 cc., of left 495 cc. Water equivalent of calorimeters with contents, R 3505, L 3491. Rectal temperature 37.00°C. At the end of the experiment he walked quite straight along a crack. Later the dizziness went on increasing and he still felt it after two hours.

John R. Examination of effect of alcohol upon the bloodflow. Hands in bath at 1.25 p.m., in calorimeters at 1.35, out of calorimeters

at 2.21. Pulse at beginning of observation 60, in two observations. The day was muggy. At 1.47 p.m., he got 70 cc. of port wine; at 1.51, 35 cc.; at 1.58 p.m., 70 cc. more. At 2.09, he got 20 cc. of whisky diluted with an equal volume of water. He did not like the whisky as well as the wine. He said it tasted bad.

TIME	R	L	ROOM	TIME	R	L	ROOM	NOTES
1.34½	31.700	31.690		1.59	33.10	32.740	25.1	Feels warm "inside."
1.36	31.720	31.720	24.7	2.00	33.17	32.770		Slightly dizzy.
1.37	31.790	31.750	24.9	2.01	33.23	32.830	25.1	
1.38	31.860	31.800		2.02	33.29	32.870		
1.39	31.925	31.855	25.1	2.03	33.35	32.920		Pulse 68.
1.40	32.000	31.900		2.04	33.40	32.970		
1.41	32.070	31.950	25.15	2.05	33.43	32.990		Dizziness increasing.
1.42	32.130	32.000	25.1	2.06	33.48	33.030	25.0	
1.43	32.210	32.050		2.07	33.51	33.060		
1.44	32.270	32.080	25.0	2.08	33.56	33.100		
1.45	32.320	32.110		2.09	33.60	33.140		
1.46	32.380	32.160	24.8	2.10	33.63	33.155	25.1	
1.47	32.460	32.210		2.11	33.67	33.190		Sleepy; increasing dizziness.
1.48	32.485	32.240		2.12	33.73	33.240		
1.49	32.530	32.280		2.13	33.78	33.285	25.1	Pulse 69.
1.50	32.610	32.340	24.9	2.14	33.82	33.340		Dizziness increasing.
1.51	32.690	32.390		2.16	33.90	33.410		
1.52	32.720	32.430	25.0	2.17	33.94	33.440	25.0	
1.53	32.780	32.460		2.18	33.98	33.460		
1.54	32.830	32.520		2.19	34.02	33.490	25.0	Dizziness constantly increasing. No other effect
1.55	32.880	32.555	24.9	2.20	34.06	33.525		
1.56	32.950	32.600	24.9	2.21	34.09	33.550		
1.57	33.000	32.655		2.27	34.00	33.460		Walks straight on floor.
1.58	33.060	32.690						

Cooling of calorimeters in 6 minutes 0.09°C. Volume of right hand 410 cc., of left 383 cc. Water equivalent of calorimeters with contents, R 3423, L 3391. Pulse 54. Rectal temperature 36.65°C.

Otis S. Hands in bath at 3.01 p.m., in calorimeters at 3.10½, out of calorimeters at 3.31. At 3.21 he received 3ii of whisky and then a glass of water.

TIME	R	L	ROOM	TIME	R	L	ROOM
3.09	31.730	31.700	29.45	3.22	32.235	32.350	29.8
3.12	31.800	31.820	29.90	3.23	32.270	32.390	
3.13	31.840	31.860		3.24	32.300	32.430	
3.14	31.895	31.920	29.80	3.25	32.340	32.470	29.8
3.15	31.950	31.980		3.26	32.380	32.510	
3.16	32.000	32.040	29.90	3.27	32.415	32.545	
3.17	32.060	32.120		3.28	32.455	32.590	
3.18	32.090	32.160		3.29	32.495	32.630	
3.19	32.130	32.210	29.80	3.30	32.520	32.660	29.9
3.20	32.170	32.260		3.31	32.570	32.725	
3.21	32.210	32.325		3.39	32.510	32.835	

Cooling of calorimeters 0.06 in 8 minutes. Rectal temperature 37.8°. Volume of right hand in calorimeter 543 cc., of left hand 570 cc. Water equivalent of calorimeters with contents, R 3429, L 3451.

STUDIES ON THE VASOMOTOR CENTRE
XVIII. THE EFFECTS OF VERATRUM VIRIDE AND
CEVADIN (VERATRIN)

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INTRODUCTION

Veratrum and cevadin (veratrin), although distinct in many of their actions, produce practically identical effects on the circulation. Administering the drugs by vein, we have been able to confirm the result of Bezold and Hirt (1), Lissauer (2), and H. C. Wood, Jr. (3). The most conspicuous effect on normal animals is a sharp but brief fall of blood pressure associated with great slowing or temporary arrest of the heart. This is due to stimulation of the vagus centre, for it does not occur if the vagi are divided. The vagus centre being excluded in this way, the heart is not slowed, and the blood pressure may show either a slight rise or slight fall.

The vasomotor centre, we find, is often unaffected, and sometimes moderately stimulated; but this stimulation is wholly indirect. It is due partly to the fall of blood pressure, and partly to the respiratory embarrassment; for it does not occur if these are excluded by section of the vagi and artificial respiration.

The respiratory changes to which we have just alluded are rather conspicuous and consist in a brief depression or arrest of respiration, but with large doses the depression may be persistent. The respiratory depression and the fall of blood pressure occur fairly synchronously but the vasomotor action follows somewhat later.

METHODS (4)

Dogs were used under morphine-ether anesthesia; curare was not employed. The spleen was the organ perfused. The respiratory tracings were taken from a tracheal cannula. The drugs were always administered by vein in normal saline solution.

VERATRUM VIRIDE

This was given by vein in doses of 2.5 to 10 mgm. of the fluid extract per kilogram to twelve dogs. When the *vagi* were *intact* the drug caused a prompt, sharp fall in blood pressure from cardiac slowing, with return to the normal in a few minutes (fig. 1); with repeated dosage there is a large rise in pressure. The fall in pressure was rather variable, from 35 to 100 mm. The vasomotor centre was stimulated slightly but three times in nine injections of the smaller dosage and not at all from two injections of 10 mgm. With *divided vagi* the centre was stimulated but once in three experiments. The blood pressure fell slightly as a rule. In other experiments repeated dosage caused the pressure to rise greatly but in these the centre was not active. The cause of the rise in pressure in such experiments is not clear; it does depend upon central vasomotor stimulation for Wood (3) found that it occurred when the centre was paralyzed by section of the cord; increased heart rate partially explains it.

CEVADIN

With the *vagi intact*, doses of 0.025 and 0.05 mgm. per kilogram were sufficient to cause a severe, sudden fall in pressure quite similar to that of veratrum but greater. These doses, in six of ten injections, resulted in a brief, moderate excitation of the vasomotor centre, fairly synchronous with the lowering of the pressure (fig. 2). The fall in pressure was about the same whether the centre was stimulated or not. Repeated doses were not given with intact *vagi*.

When the *vagi* were *divided* similar quantities stimulated the centre somewhat in three of six cases. The blood pressure rose



FIG. 1.

FIG. 1. VERATRUM VIRIDE ON VASOMOTOR CENTRE AND BLOOD PRESSURE.

Experiment 44. Dog; Spleen perfused; vagi intact. Upper curve, carotid blood pressure from membrane manometer. Middle curve, carotid pressure from damped mercury manometer. Signal line, zero of blood pressure. Lowest line, outflow in units. At 5 Veratrum 10 mg. per kilogram intravenously. Note the decrease in heart rate causing the fall in pressure; outflow scarcely affected.

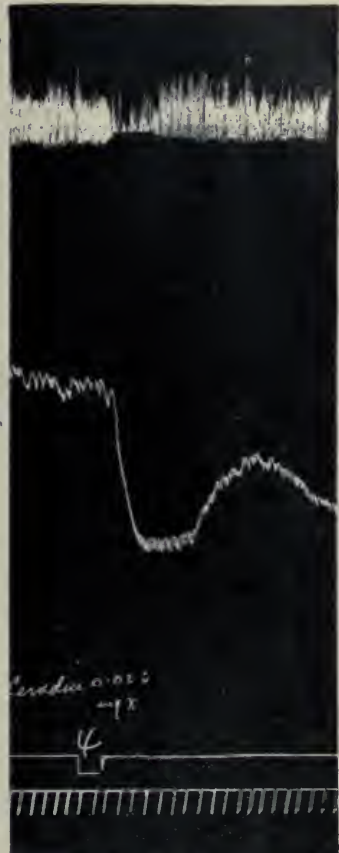


FIG. 2.

FIG. 2. CEVADIN ON THE VASOMOTOR CENTRE, BLOOD PRESSURE AND RESPIRATION.

Experiment 234. Dog; spleen perfused; vagi intact. Upper curve, respiratory tracing from the trachea. Middle curve, mean carotid pressure from damped mercury manometer. Signal line, zero blood pressure. Lowest line, outflow in units. At 4 cevadin 0.025 mg. per kilogram intravenously. Note the temporary cessation of respiration, fall in blood pressure and slight temporary decrease in the outflow.

in five of them, a mean of 15 mm. (extremes 7 and 65 mm.), and fell 20 mm. in the exceptional case.

One animal showed slight convulsive phenomena after each of three injections of cevadin and each time there was central vasomotor stimulation.

The circulatory action is not secondary to respiratory depression for cevadin causes the fall in pressure and cardiac slowing under artificial respiration as illustrated in an experiment in which the fall in pressure was even slightly greater under artificial respiration.

ACTION ON RESPIRATION

During the earlier experiments no especial attention was paid to the respiratory action, although several times irregular, gasping respiration was noted, more often in the experiments with stimulation of the vasomotor centre. The respiration was then recorded in four experiments with cevadin (0.025 mgm. per kilogram). In three cases the respiration was immediately stopped and in the fourth considerably depressed. The respiratory action was synchronous with the fall in blood-pressure and gradually returned to the normal as the pressure rose to the normal (fig. 2). In three of the experiments the vasomotor centre was stimulated somewhat.

Veratrum also caused a similar but possibly somewhat lesser action in eight experiments with dosage of 2.5 to 5 mgm. per kilogram. Occasionally repeated dosage did not further depress respiration. After atropin and section of the vagi there was usually less respiratory action, although there are exceptions to this statement. Respiration may be depressed when there is no action on the blood pressure.

THE CAUSE OF THE VASOMOTOR ACTION

The data show that veratrum in the doses used seldom affects the vasomotor centre, and that cevadin stimulates the centre regardless of the effect on blood pressure in a little more than half the experiments. The central stimulation might be attrib-

uted to anemia from the fall in pressure and this undoubtedly plays a part and would probably have had a more pronounced action had the pressure not returned so promptly to the normal. However the centre was also stimulated in half the experiments after section of the vagi, when the blood pressure may show either a moderate rise or fall. The chief factor in the vasomotor excitation is the asphyxia from the respiratory depression and occasionally, also, from the direct action when convulsions occur.

The maintenance of artificial respiration during the action of veratrum prevents the vasomotor action, as occurred several times in experiment B 8: The action of the first dose of 5 mgm. per kilogram was not observed because of a technical error; the second dose caused a marked rise in pressure and considerable central vasomotor stimulation, which was relieved by artificial respiration. Two following doses during artificial respiration each decreased the central tone somewhat as the pressure rose considerably; in each instance, also, when the artificial respiration was discontinued the central tone was increased. The period of greater vasomotor tone was observed to be synchronous with the period of increased venosity of the arterial blood from the respiratory depression; when artificial respiration was performed the blood assumed a brighter color and the vasomotor tone was lessened, a phenomenon that was observed several times. Respiratory embarrassment was noted most frequently with cevadin in those experiments in which the centre was stimulated.

Doubtless, then, the combination of anemia from the fall in blood pressure and the asphyxia from the respiratory depression frequently result in excitation of the vasomotor centre, but as both pressure and respiration soon return to the normal, the centre may be unaffected, and at best the effect is transitory, unless sufficiently large doses have been given to depress the respiration continuously, when the increased tone may also persist.

Attention is called to the value of veratrum and cevadin as illustrating pure central vagus stimulation. With the doses used the fall in blood pressure depends largely upon a marked decrease in heart rate (fig. 1) which does not occur after vagotomy.

CONCLUSIONS

Veratrum viride and the related alkaloid cevadin are without direct action on the vasomotor centre. As a result of anemia from the fall in blood pressure, or from asphyxia from respiratory depression, the centre may be stimulated in about half the experiments; such action may be prevented by artificial respiration.

Convulsant doses of cevadin also increase the central vasomotor tone.

REFERENCES

- (1) BEZOLD AND HIRT: quoted from Lissauer (2).
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A PHYSIOLOGICAL TEST FOR CHOLIN AND SOME OF ITS APPLICATIONS

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The suggestion has frequently been made in recent years that certain symptoms classed as "vagotonic" (miosis, salivation, bradycardia, pylorospasm, etc.) may be due to the presence of abnormal amounts of cholin. It has also been suggested that cholin or some derivative of it may be a factor in certain physiological processes; that, for example, such compounds may function as hormones for the parasympathetic nervous system in the same way that epinephrine is believed to function as a hormone for the sympathetic nervous system. The discovery that certain comparatively simple compounds of cholin (compounds which might conceivably easily be formed in the body), are extraordinarily active¹ has especially directed attention to this possibility.² The discovery of the presence of considerable amounts of cholin in the suprarenal glands³ early led to the suggestion that these glands are connected in some special manner with the occurrence or metabolism of cholin in the body. Cholin has been reported as being present in increased amounts in the blood and cerebrospinal fluid in certain disorders accompanied by extensive destruction of nervous tissue. Much emphasis has been laid by some writers upon a supposed involve-

¹ Hunt and Taveau: *British Medical Journal*, 1906, ii, 1788; *Bulletin* 73 U. S. Hygienic Laboratory, 1911.

² Hunt and Taveau: *Journ. of Pharm. and exp. Therap.*, 1909, 1, 303; Dale, *ibid.*, 1914, 6, 149.

³ Marino-Zucco: *Maly's Jahresbericht*, 1888, 18, 231; Hunt: *Amer. Jour. Physiology*, 1899, 3, p. xviii; 1901, 5, p. vi; Lohmann: *Pflüger's Archiv*, 1907, 118, 215.

ment of cholin in the action of the Roentgen rays upon tissues, especially upon neoplasms.

Many of these suggestions are purely hypothetical and based only upon analogy; others were based upon chemical tests the validity of which, under the conditions in which they were used, have been called into question. It has been impossible to test experimentally some of the other questions raised owing to the lack of methods by which minute amounts of cholin occurring in the blood or other fluids or tissues could be detected. The lack of such a method is largely responsible for the very incomplete knowledge of the history of cholin in the body, how it is absorbed, its fate, etc.

In 1906 Taveau and I¹ proposed two new methods for the detection of small amounts of cholin which we considered better than those then in use. One of these methods was chemical and consisted in converting the cholin into the benzoyl derivative which formed a compound with platinum chloride which could, apparently, be easily distinguished from other platinum compounds likely to be formed under the conditions. The other method proposed was physiological and consisted in converting the cholin into the acetyl derivative whereby the physiological activity was increased many thousand times. The present communication deals with this physiological test.

METHOD

✓ As was stated above the method proposed consists in converting the cholin into the acetyl derivative (by heating the cholin with acetyl-chloride in a sealed tube in the water bath) and testing the physiological action of the reaction product. Details as to the method as applied to various fluids and organs will be given below; here a few words will be said as to the physiological tests employed.

- ① In the earlier experiments the effect upon the blood-pressure of the acetylated product was determined. Acetyl-lecholin has an extraordinary power of lowering the blood-pressure due chiefly, according to my experiments, to an action upon the heart; as

little as 0.000005 mgm. and even less may cause a distinct fall of blood-pressure when injected intravenously into a rabbit or cat. This action is prevented by atropine. The blood-pressure method is thus sufficiently sensitive to detect minute amounts of cholin; it has also the advantage that the effect is of very brief duration and tests may be made in rapid succession. A serious disadvantage of the blood-pressure method is that, although it is so sensitive, it does not readily permit of the quantitative determination of small amounts of acetyl-cholin: that is, there is often not a very great difference between the effects of a small amount of acetyl-cholin and an amount several times greater. Thus the following results were obtained upon a cat: 1 cc. of the blood serum of a cat was treated according to the method to be described below; 1 cc. of the acetylated product in a dilution of 1 in 100 caused the blood-pressure to fall 20 mm. mercury (19.2 per cent) whereas 1 cc. of a dilution 1 in 40 caused the pressure to fall 30 mm. mercury (28.8 per cent). A difference of 50 per cent in the concentration of such solutions could scarcely have been detected.

Attention was next directed to the frog's heart as a test for acetyl-cholin. A solution of acetyl-cholin 1 in 200,000,000 perfused through a frog's heart by way of the vena cava promptly diminished the amplitude of the beat and usually caused the heart to stop for considerable periods in diastole. It was soon evident, however, that this method was far more sensitive than was necessary for my purposes and in subsequent experiments the "Straub Method,"⁴ with slight modification, was employed. The modification consisted in the use of a cannula with a side tube very near the heart through which the fluid was easily removed and fresh fluid introduced thus permitting of frequent and thorough renewal of the fluid. The hearts prepared in this manner seemed to be less sensitive than the hearts perfused through the vena cava; no careful studies, however, were made upon this subject. The Ringer's solution usually employed was made according to Clark's formula.⁵

⁴ Cf. Fühner: Nachweis und Bestimmung von Giften auf biolog. Wege, p. 123.

⁵ Clark: Journ. of Pharm. and exp. Therap., 1913, 4, 403.

The hearts employed varied considerably in their susceptibility to acetyl-cholin, and their susceptibility diminished, often greatly, in the course of a long experiment; usually 1 part in from 50 to 60 millions was sufficient to bring the heart to a standstill of several seconds duration early in an experiment. Since 0.5 cc. of a solution was sufficient for a test it is evident that under favorable conditions, as little as 0.00001 mgm. of cholin could be detected; as a matter of fact I have taken 0.0001 mgm. of cholin, acetylated it, and obtained sufficient solution for many tests and from 1 cc. of serum sufficient acetyl-cholin could often be obtained to have made a thousand tests.

Differences of only 10 per cent were frequently detected in this manner; usually, however, no effort was made to detect such small differences.

Different hearts differed in their response to acetyl-cholin; in some cases the effect was chiefly inotropic, in others almost wholly chronotropic, more usually a combination of the two. This variation in response made the comparison of solutions of different strength, based on the intensity of the effects produced, difficult. Accordingly efforts were made to adjust the solutions so that their effects presented the same general picture; this could only be done by trying solutions of different strengths, which was a very time-consuming process often involving the use of several hearts. Such careful comparisons were not as a rule necessary for my purpose and only a few were made.

After very small amounts of atropine far greater concentrations of acetyl-cholin are necessary to produce any effect upon the heart. It was thus possible to compare solutions of the same relative, but of very different absolute, strengths; comparisons of this character gave very concordant results.

Curves illustrative of many of the above points will be introduced in later parts of this communication. Attention may be called here to figure 1 showing the difference between the action of cholin and¹ of acetyl-cholin upon the frog heart: the acetyl-cholin was prepared from a solution containing 0.1 mgm. cholin. As will be seen the acetyl-cholin 1 in 35,000,000 and 1 in

40,000,000 had a greater effect than the cholin 1 in 1000; that is the acetyl-cholin was more than 40,000 times as active as the cholin.

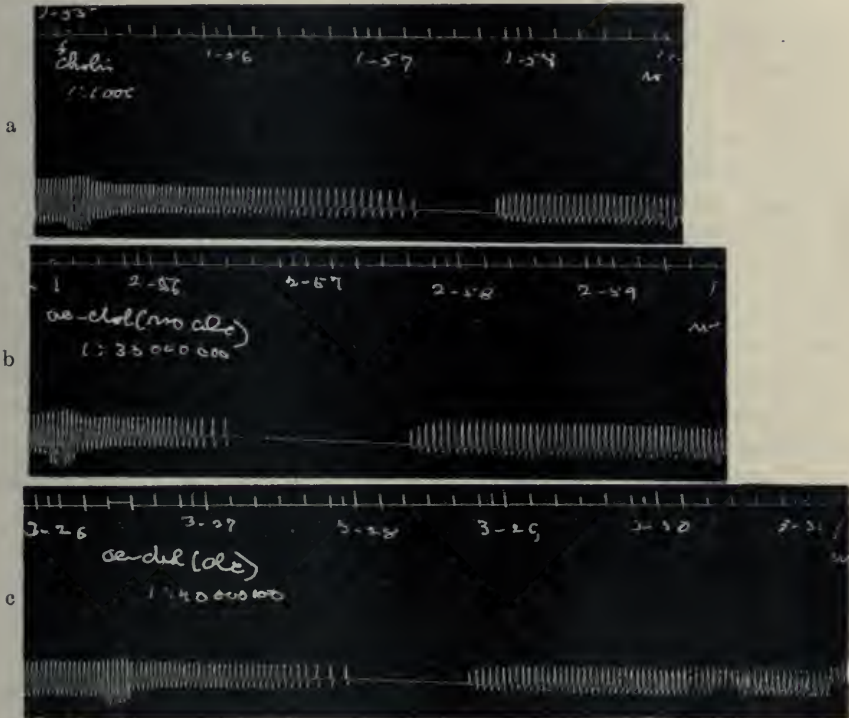


FIG. 1. FROG HEART; MAY 28; STRAUB METHOD

Tracings read from left to right. Time in minutes and 10 seconds. The signal indicates where the Ringer's solution was replaced by the cholin solution; "w" indicates that the cholin solution was replaced by pure Ringer's solution.

- a. 1-55. Cholin 1 in 1000.
- b. 2-56. Acetyl-cholin 1 in 35,000,000.
- c. 3-26. Acetyl-cholin 1 in 40,000,000.

The acetyl-cholin 1 in 40,000,000 had been treated with alcohol; that 1 in 35,000,000 had not.

A few words may be said here concerning a possible source of error in the preparation of the acetyl-cholin. In nearly all of the experiments the excess of acetyl-chloride in the acetyla-

tion tube was removed by evaporation at about 50°C. under diminished pressure. Then, in order to remove the last traces of the chloride, the reaction product was dissolved in a small amount of absolute alcohol and evaporated to dryness; in some cases this was done twice. After this method had been employed for some time the communication of Fourneau and Page⁶ came to my attention; these authors speak of the danger of decomposing acetyl-cholin chloride when it is treated with alcohol. Further experiments, however, in which the same amounts of cholin (0.1 mgm. in each case) were treated with acetyl-chloride and the reaction product in the one case was dissolved in alcohol three times and the alcohol evaporated each time and the residue dissolved in Ringer's solution and, in the other case, in which the excess of acetyl-chloride was removed by a more prolonged evaporation, under diminished pressure, and the product then dissolved in Ringer's solution, gave solutions of equal activity, (see fig. 1). Hence any loss of acetyl-cholin from the alcohol treatment was too slight to be detected. In fact acetyl-cholin in solution in absolute alcohol could be kept for considerable time without there being an appreciable loss of activity. In aqueous or in Ringer's solution, however, acetyl-cholin lost its activity somewhat rapidly as Taveau and I found some time ago.

Reference may be made to attempts to detect small amounts of cholin by its conversion into pseudomuscarin (nitrous acid ester of cholin)⁷ by treatment of the platinum salt with concentrated nitric acid. It seemed possible that by this method substances which might interfere with the physiological tests would be destroyed. As a matter of fact it was possible to prepare from 0.01 mgm. cholin sufficient of the nitrous acid ester to give satisfactory tests. But the method is far less delicate than the acetyl-cholin method; a solution of the nitrous acid ester 1 in 100,000⁸ was less active than a solution of acetyl-

⁶ Fourneau and Page: Bull. Soc. Chim., 1914, 15, 544.

⁷ Ewins: Biochem. Jour., 1914, 8, 209; Dale and Ewins: Jour. of Physiol., 1914, 48, p. xxiv.

⁸ Fühner: Arch. exp. Path. u. Pharm., 1909, 61, 283, found that pseudomuscarin could not readily be detected in solutions weaker than about 1 in 100,000.

cholin 1 in 30,000,000. The method, moreover, was but little if any less troublesome than the acetyl-cholin method and the study of it was not pursued farther; in some cases, however, it might be a useful method especially for obtaining confirmatory evidence of the presence of cholin in complex mixtures.

✓ DETECTION OF CHOLIN IN BLOOD SERUM

In order to detect cholin in blood serum and other fluids containing protein, salts and perhaps lecithin the following method was employed; the accuracy of it was tested by determining to what extent added cholin could be recovered. One cubic centimeter of the blood serum was slowly dropped into 4 cc. of pure acetone in a centrifuge tube. After standing, tightly corked, for a few hours or over night, with occasional shaking, the solution was centrifugalized; the precipitate was washed twice with 1 cc. of acetone and centrifugalized. The acetone solution was transferred to a centrifuge tube, made faintly acid to litmus with HCl and the acetone drawn off, at a temperature of about 50°C. with the pump.² The residue was extracted with absolute ether and the ether, after centrifugalizing, pipetted off. A mixture of 0.1 cc. absolute alcohol and 0.9 cc. acetone was added to the residue; it was centrifuged and the liquid pipetted from the salts, etc., into a small tube with a bulb. The salts were extracted three times in this manner. The alcohol and acetone were completely evaporated under diminished pressure, a few drops of acetyl-chloride added, the tube sealed and heated in the boiling water bath for two hours. The tube was opened and the excess of acetyl-chloride drawn off with the pump at about 50°C. In most of the experiments 1 or 2 cc. of absolute alcohol were added and the alcohol evaporated on a warm bath; the residue was dissolved in Ringer's solution. In some cases the treatment with alcohol was omitted and the acetylation product, after exposure for a considerable time to a low pressure at 50°C., was dissolved in Ringer's solution. No difference in the activity of the solutions obtained by these two methods was noted (see above).

It was hoped by the above process to avoid the splitting off of cholin from the lecithin of the serum. Whether this object was fully attained must be left undecided. It is evident however that at most there was only a small decomposition of lecithin. The acetylation product I obtained from the blood serum of various normal animals had an activity corresponding to 1 mgm. of cholin in from about 100 to 400 cc. If the figure 0.2 per cent be accepted as representing approximately the phosphatid content of serum and assuming that this consists largely of lecithin, there could be obtained from this about 30 mgm. of cholin, that is, the cholin I obtained would correspond to less than 3 per cent of what could be obtained from the lecithin of the serum. It was moreover remarkable that such constant results should have been obtained with different portions of the same serum. Even if it should be proved that the small amounts of cholin which were obtained from normal serum resulted from the decomposition of lecithin the method would still answer the question for which it was devised, namely, is there, as has often been supposed, a marked accumulation of cholin in the serum in certain experimental or pathological conditions.

That the substance giving the physiological tests was in reality acetyl-cholin is rendered highly probable by its method of for-

FIG. 2. EXPERIMENT JANUARY 27, 1915; FROG'S HEART; STRAUB METHOD. Acetyl-cholin obtained from the following:

"S (N)," serum of normal cat; "S (O)," serum of cat after removal of suprarenals. "S (N) and cholin," and "S (O) and cholin," serum to 1 cc. of which 0.01 mgm. cholin had been added.

The acetyl-cholin was obtained by the acetylation of 0.01 mgm. cholin. Time in minutes and 10 seconds.

- a. 1-06. "Acetyl-1:10,000,000" = Acetylated cholin 1 in 10,000,000.
- b. 1-27. "S (N) 1:70" = the acetylated product of 1 cc., serum (N) diluted to 70 cc.
- c. 1-42. "S (O) 1:70" = the acetylated product of 1 cc. serum (O) diluted to 70 cc.
- d. 2-05. "S (N) and cholin 1:150" = the acetylation product of S (N) to 1 cc. of which 0.01 mgm. cholin had been added; diluted to 150 cc.
- e. 2-27. "S (O) and cholin 1:150" = the acetylation product of S (O) to 1 cc. of which 0.01 mgm. cholin had been added; diluted to 150 cc.
- f. 2-52. Showing effect of acetylated cholin 1 in 15,000,000.
- g. 3-11. Showing effect of the acetylated product of S(O) diluted to 1 in 150.

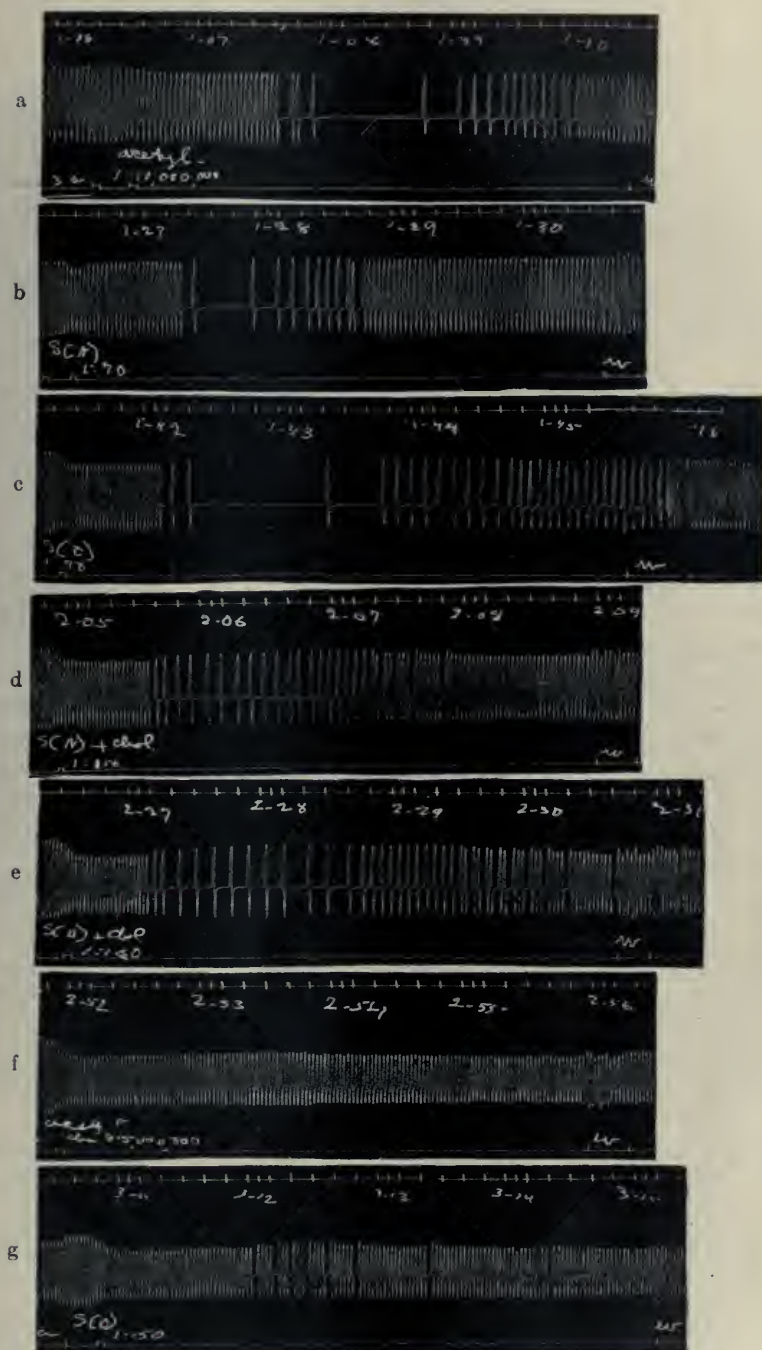


FIG. 2.

mation, the nature of the action, the fact that the action is prevented by atropine and especially by the fact that when cholin was added to the serum the activity of the reaction product was increased directly in proportion to the amount of cholin added; that is, the action of the two was strictly additive. No substance other than cholin is known which gives these results upon treatment with acetyl-chloride.⁹ In no case did serum alone, even when undiluted, give similar results.

That all, or practically all, of the cholin occurring in blood serum may be recovered by this process is shown by the following experiments, which are introduced partly to illustrate other points (see later).

Recovery of cholin added to blood serum.—Experiment January 27, 1915, (fig. 2). To 1 cc. of each of 2 specimens of cat serum "S (O)" and "S (N)," there was added 0.01 mgm. cholin; these were then treated as described above and the products of acetylation dissolved in 10 cc. of Ringer's solution. From these solutions "S (N) and cholin" and "S (O) and cholin," which correspond to solutions of acetyl-cholin 1 in 1,000,000 plus the acetyl-cholin derived from 1 cc. of serum, various dilutions were prepared. The activity of these were compared with that of dilutions of 0.01 mgm. of acetylated cholin and of the acetylation products of serum alone. From the tracings in figure 2 it is evident that

- S (N) 1 in 70 is slightly less active than acetyl-cholin 1 in 10,000,000 (comparison of a and b);
- or S (N) is slightly less active than acetyl-cholin 1 in 143,000;
- that S (O) 1 in 70 is slightly more active than acetyl-cholin 1 in 10,000,000 (comparison of a and c);
- or S (O) is slightly more active than acetyl-cholin 1 in 143,000;
- that Acetyl-cholin 1 in 15,000,000 and S (O) 1 in 150 separately had very little effect (f and g);
- that the latter combined are much more active than either taken separately, that is, that the larger part of the added cholin had been recovered (e).

Experiment December 10, 1914 (fig. 3). To 1 cc. of each of two specimens of cat serum, "Ser (N)" and "Ser (H)" there was added

⁹ It may be added that control experiments with creatin and cholesterin gave purely negative results.

0.05 mgm. cholin; these were treated as described above and the products of acetylation dissolved in 10 cc. Ringer's solution. From these solutions "Chol. and Ser (H)" and "Chol and Ser (N)" which correspond to solutions of acetyl-cholin 1 in 200,000 plus the acetyl-cholin derived from 1 cc. of serum, various dilutions were prepared. The activity of these was compared with that of solutions of 0.05 mgm. of acetylated cholin and of the acetylation products of serum alone. The susceptibility of the heart had diminished greatly when these tracings were taken.

From the curves of figure 3 it is seen that:

Ser (H) 1:40 is slightly more active than acetyl-cholin 1 in 3,000,000 and less active than acetyl-cholin 1 in 2,000,000 (d, a and c).

Or Ser (H) is less active than acetyl-cholin 1 in 50,000 and more active than acetyl-cholin 1 in 75,000.

That cholin 1 in 3,000,000 plus S (H) 1 in 150 and also cholin 1 in 3,000,000 plus S (N) 1 in 150 is about equal to acetyl-cholin 1 in 2,000,000 or that the greater part of the added cholin was recovered (e and c).

Experiment December 4, 1914 (fig. 4). To 1 cc. of guinea-pig serum there was added 0.025 mgm. cholin; the acetylation product was dissolved in 10 cc. Ringer's solution. From this solution which corresponds to a solution of acetyl-cholin 1 in 400,000 plus the acetyl-cholin derived from 1 cc. of serum, various dilutions were prepared and their activity compared with that of solutions obtained by acetylating 0.05 mgm. cholin and of the acetylation product of 1 cc. of serum.

The tracings in figure 4 show that:

Serum (acetylated) 1:100 is about = acetylated cholin 1 in 8,000,000 (c and b);

Or that serum (acetylated) is about = acetyl-cholin 1 in 80,000;

And that cholin 1 in 16,000,000 plus serum (acetylated) 1 in 400 is slightly more active than either; that is more cholin seems to have been recovered than was added or that some of the cholin in the control or in the acetylated serum had been lost (a, b and c).

The experiment of March 3, 1915 (fig. 11) also shows that cholin added to serum may be recovered.

From the above and similar experiments it appears that from 80 to 100 per cent of cholin added to serum may be recovered; hence it may be assumed that cholin occurring in a free condition in the serum may be obtained by the above process with a fair degree of accuracy considering the small amounts of cholin

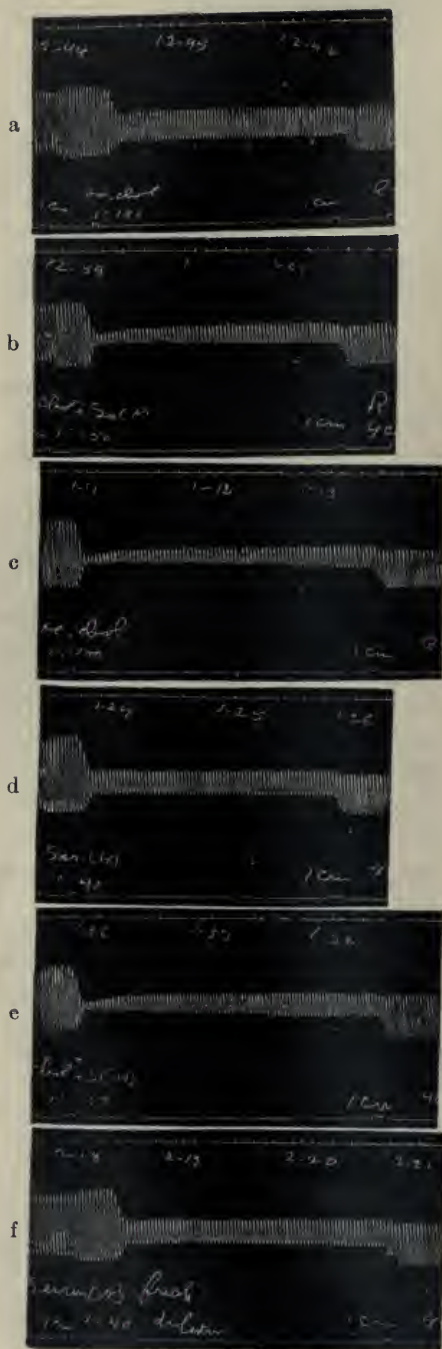


FIG. 3. EXPERIMENT DECEMBER 10, 1914. FROG'S HEART; STRAUB METHOD

Ser (H) was cat's serum heated to 55-59°C. for 1½ hours

Ser (N) was cat's serum which had not been heated.

"Cholin and Ser (N)" and "Cholin and Ser (H)" were prepared by adding 0.05 mgm. cholin to 1 cc. of the "Ser (N)" and "Ser (H)" and after about 13 hours precipitating with acetone, acetylating, etc.

The acetyl-cholin was prepared by acetylating 0.05 mgm. cholin.

a. 12-44. "Acetyl-cholin 1:150" = acetylated cholin 1 in 3,000,000.

b. 12-59. "Cholin and Ser (N) 1:150" = acetylated cholin 1 in 3,000,000 plus acetylated Ser (N) 1 in 150.

c. 1-11. "Acetyl-cholin 1:100" = acetylated cholin 1 in 2,000,000.

d. 1-24. "Ser (H) 1:40" = acetylated Ser (H) 1 in 40.

e. 1-36. "Cholin and Ser (H) 1:150" = acetylated cholin 1 in 3,000,000 plus acetylated Ser (N) 1 in 150.

f. 2-18. "Ser (N) 1:40" = acetylated Ser (N) 1 in 40.

involved and the limitations of accuracy in most physiological tests.

Is cholin destroyed by blood serum? In some of the earlier experiments of this series results were obtained (probably as a

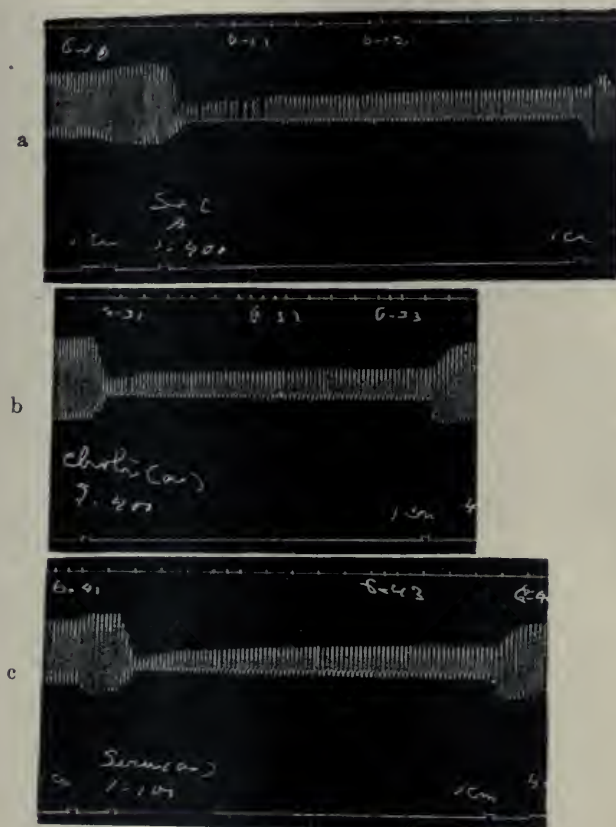


FIG. 4. EXPERIMENT DECEMBER 4, 1914. FROG'S HEART; STRAUB METHOD

Guinea-pig serum, precipitated with acetone, acetylated, etc. To 1 cc. of the serum 0.025 mgm. cholin has been added, the solution treated with acetone, etc. The acetylated cholin was made from 0.05 mgm. cholin.

a. 6-10. "S and C. A. 1:400" = acetylated cholin 1 in 16,000,000 plus the acetyl-cholin derived from 1 cc. serum, diluted to 400.

b. 6-31. "Chol (ac) 1:400" = acetylated cholin 1 in 8,000,000.

c. 6-41. "Serum (ac) 1:100" = the acetylation product of 1 cc. serum diluted to 100.

result of faulty technique) which suggested that when cholin was added to fresh serum and the solution allowed to stand for some time a part of the cholin was destroyed. Thinking that possibly a ferment action was involved the experiment described above (experiment December 10; see figure 3) was performed. As the results show there was no difference between the amounts of cholin recovered from the solution in the heated and in the unheated serum. Comparison of the effects of the acetylation products of the heated and of the unheated serums showed that an hour and a quarter's heating of serum at 55° – 59°C . had not led to an increase in the free cholin of the former and that standing for 13 hours, part of the time at 37°C ., had not led to an increase of cholin in the unheated serum.

Recovery of cholin when added to muscle. In connection with experiments to be described later it was desirable to know if the above method was applicable to muscle; hence the following experiment was performed.

Experiment February 5, 1915. Guinea pig, male, 538 gm. Suprarenals removed 10.30 a.m.; in afternoon hair rough and animal quiet. Died between 14 and 22 hours after removal of suprarenals. Two portions of muscle, 1 gm. each, were removed from the abdominal muscles; into one ("Op. muscle") was injected, with a hypodermic syringe 0.1 cc. absolute alcohol, into the other ("Op. muscle and cholin") 0.1 cc. of an alcoholic solution of cholin containing 0.01 mgm. cholin. A normal, control, guinea pig, male, 600 gm., was kept without food

FIG. 5. EXPERIMENT FEBRUARY 5, 1915. FROG'S HEART; STRAUB METHOD

Time in minute and 10 seconds; "w" indicates that the heart was washed out with fresh Ringer's solution.

a. 2-49. "Op. mus. and chol. 1:1500" = acetylated cholin 1 in 150,000,000 plus the acetyl-cholin obtained from 1 gm. of muscle of the adrenalectomized guinea pig and diluted to 1500 cc.

b. 3-05. Acetyl-cholin 1 in 100,000,000.

c. 3-34. Acetyl-cholin 1 in 60,000,000.

d. 3-51. "N. muscle 1:800" = the acetylated product of 1 gm. normal muscle, 1 in -800.

e. 4-11. "Op. muscle 1:800" = the acetylated product 1 in 800 of 1 gm. of muscle from the adrenalectomized guinea pig.

f. 4-46. "Serum 1:300" = the acetylated product of 1 cc. normal guinea pig serum diluted to 300 cc.

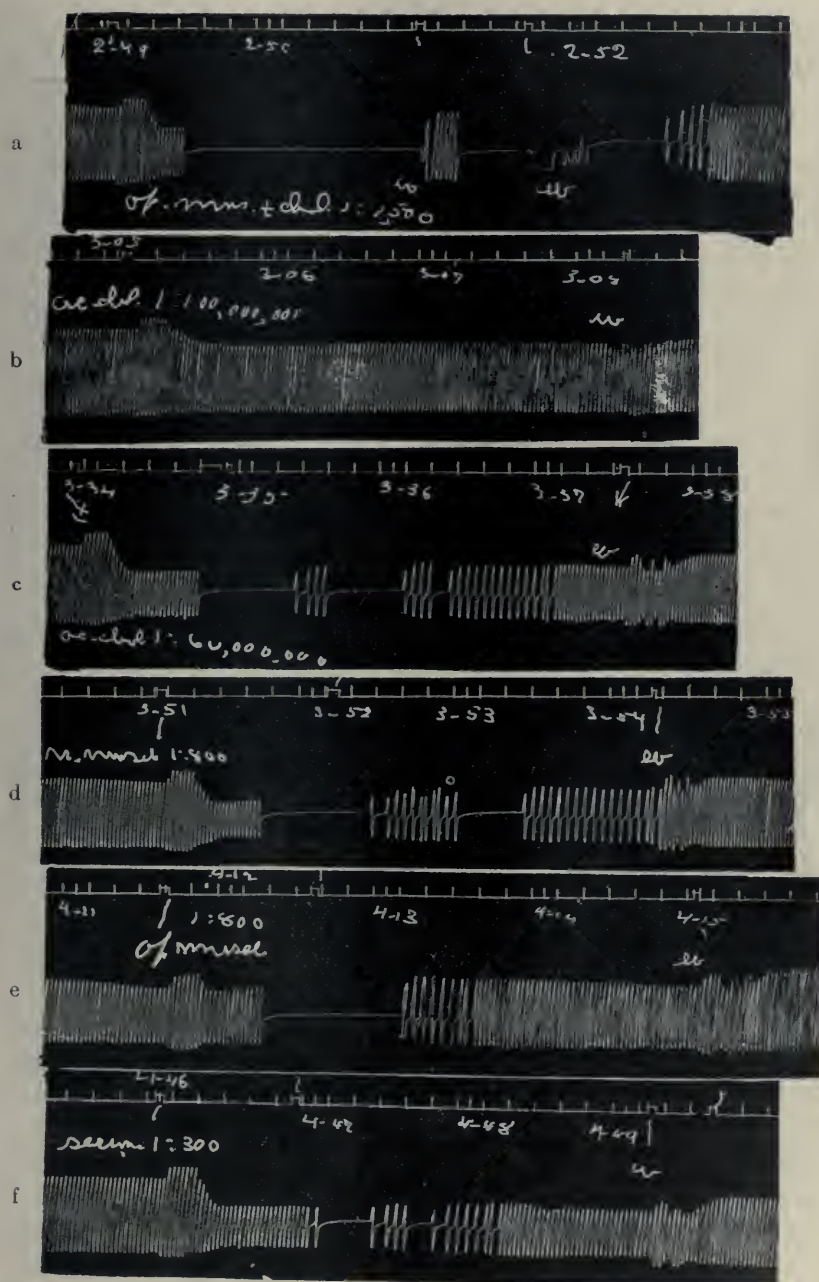


FIG. 5

for 22 hours when it was bled from the heart; the blood was defibrinated and centrifugalized. One gm. of muscle was removed (this the "N muscle"). The samples of muscle were placed in 4 cc. portions of acetone for two hours; they were then ground with sand and replaced in the acetone and allowed to stand over night. After being again ground with sand they were centrifugalized, and the residues were extracted twice with 1 cc. portions of acetone. The acetone extracts were treated as in the case of serum and acetylated. One cc. of the serum of the normal guinea pig was treated in the usual way and acetylated. The activity of these various acetylation products was compared with that of dilutions of acetyl-cholin made by acetylating 0.01 mgr. of cholin.

The results are shown in figure 5 which shows that:

"Op. muscle" 1 in 800 is slightly less active than acetyl-cholin 1 in 60,000,000. (e and c) or "Op. muscle" is slightly less active than acetyl-cholin, 1 in 75,000.

That acetyl-cholin 1 in 150,000,000 plus the acetyl-cholin derived from 1 gm. of muscle and diluted to 1500 is more active than either of the above taken separately (a, c and e) that is, practically all of the cholin was recovered.

Numerous other comparisons with different dilutions gave similar results.

(This experiment also shows that the normal muscle contained slightly more cholin than the "operated" muscle; also that the normal muscle contained about three times as much cholin as the blood serum.)

Recovery of cholin when added to urine. To 1 cc. of the urine of the normal guinea pig of the above experiment there was added 0.01 mgr. of cholin dissolved in 0.1 cc. absolute alcohol; to another cubic centimeter of the urine there was added 0.1 cc. absolute alcohol. Both specimens were made faintly acid to litmus with hydrochloric acid, evaporated to dryness and extracted several times with alcohol. The alcohol was evaporated and the residue again extracted with alcohol; the alcoholic solution was transferred to a tube, the alcohol evaporated, acetyl-chloride added, etc., as in the case with blood serum. The acetylation product of the urine in a dilution of 1 in 300 reduced the heart beats in two minutes to 48; a similar dilution of the acetylation product of the urine containing cholin (1 in 30,000,000)

reduced it to 30 beats; acetyl-cholin alone, 1 in 30,000,000 weakened but did not slow the heart. The urine 1 in 500, plus acetyl-cholin 1 in 50,000,000 was distinctly more active than acetyl-cholin alone 1 in 30,000,000, i.e., the cholin added to the urine had been largely recovered. (Other comparisons showed that the cholin content of the urine was about twice that of the blood serum.)

Disappearance from the blood of cholin injected intravenously. As the primary purpose of the development of this method was to determine whether, as has often been suggested, there is, in certain pathological conditions, an accumulation of cholin in the blood it was of interest to determine how rapidly cholin injected intravenously disappears from the blood. If it disappears from the blood very rapidly it is evident that there might be a very great increase in the formation of cholin without there being at any one time a marked increase in the amount in the blood. In view of the fact that most substances injected intravenously do leave the blood very quickly it was to be anticipated that cholin would do the same. Hence it was scarcely to be expected that determinations of the amount of cholin in the blood would definitely answer the question whether, under certain conditions, there is or is not an increased formation of cholin and whether this may or may not be involved in the causation of certain symptoms.

Ellinger¹⁰ has recently reported experiments on the distribution of cholin in the body after its intravenous injection; from his results it would appear that cholin leaves the blood rapidly. Ellinger's method involved the prolonged extraction of the blood and tissues with hot alcohol or acid, a process which probably led to the decomposition of some of the lecithin and so an increase in the amount of cholin obtained from certain tissues. Ellinger also employed chemical tests which are scarcely sufficiently delicate to permit of the identification, with certainty, of minute amounts of cholin. Nevertheless Ellinger's results seem to show clearly that cholin injected intravenously does disappear rapidly from the blood.

¹⁰ Ellinger: Münch. Med. Woch., 1914, 61, 2336.

In order to obtain further information on the rate at and the extent to which cholin, injected intravenously, leaves the blood the following experiments were performed.

Experiment February 25, 1915 (fig. 6). Rabbit 2015 gm. The rabbit had been used for another experiment and the blood pressure had fallen to 55 mm. mercury. Ten mgn. cholin chloride dissolved in 1 cc. 0.8 per cent NaCl was injected rapidly into the right external jugular vein; 1 minute, 6 minutes and 11 minutes after the completion of the injection portions of about 3 cc. of blood were collected from the left carotid; these portions of blood were treated in the usual manner and the activity of the products of acetylation (of 1 cc. in each case) compared with that of the acetylation product of blood drawn before the injection of the cholin and with acetylated cholin.

The curves in figure 6 show the following:

1. Comparisons of g and h show that the acetylation product of normal serum 1 in 50 is comparable in activity to acetyl-cholin 1 in 20,000,000.

Or that normal serum is comparable to cholin 1 in 400,000.

2. Comparison of f and h show that serum one minute after the injection of cholin, in a dilution of 1 in 300 is slightly less active than (acetylated) cholin 1 in 20,000,000.

Or that the "1 minute-blood" was only slightly less active than cholin 1 in 66,000.

Comparisons of g, f, c and d show that "1 minute-blood" was between 4 and 6 times as active as normal serum; this conclusion is in agreement with 2.

4. Comparisons of b and c show that "1 minute-blood" is more than twice as active as "6 minute-blood."

5. Comparisons of a and c show that "1 minute-blood" is almost 5 times as active as "11 minute-blood."

From the above the following conclusions may be drawn:

1. The normal serum had approximately 1 part of cholin in 400,000.

2. The "1 minute-blood" had approximately 1 part of cholin in 66,000.

3. The "6 minute-blood" had less than 1 part of cholin in 132,000.

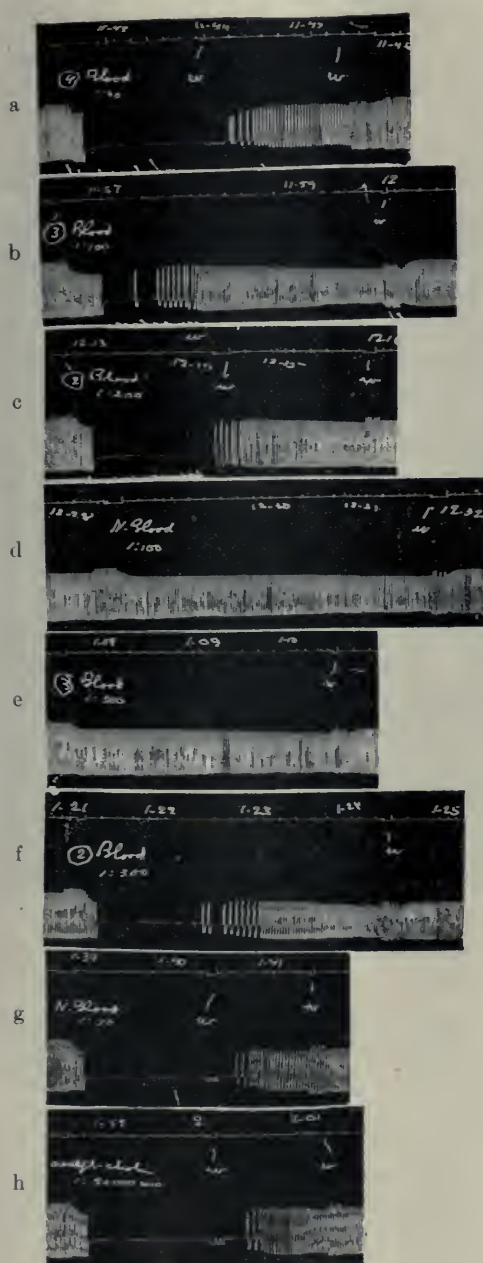


FIG. 6. EXPERIMENT FEBRUARY 25, 1915. FROG'S HEART

"w" indicates that the acetyl-cholin solution was replaced by pure Ringer's solution.

a. 11-43. "4 Blood 1:40" was the acetyl-cholin from 1 cc. of serum of the blood drawn 11 minutes after the injection of 10 mgm. cholin; dilution 1 to 40.

b. 11-57. "3 Blood 1:100" was the acetyl-cholin from 1 cc. of the serum of the blood drawn 6 minutes after the injection of cholin; dilution 1 to 100.

c. 12-13. "2 Blood 1:200" was the acetyl-cholin from 1 cc. of the serum of the blood drawn 6 minutes after the injection of cholin; dilution 1 to 200.

d. 12-28. "N. Blood 1:100" was the acetyl-cholin from 1 cc. of the serum before the injection of cholin; dilution 1 in 100.

e. 1-08. "3 Blood 1:200" same as b; dilution 1 to 200.

f. 1-21. "2 Blood 1:300" same as c; dilution 1 to 300.

g. 1-39. "N. Blood 1:50" same as d; dilution 1 in 50.

h. 1-59. Acetyl-cholin 1 in 20,000,000.

4. The activity of "11 minute-blood" was not much greater than that of normal serum or that nearly all of the 10 mgm. of cholin had disappeared from the serum in 11 minutes.

The extraordinary rapidity with which the injected cholin had disappeared from the serum is further emphasized by a comparison of the amounts of cholin found 1 minute after the injection of the 10 mgm. cholin and the amount which would have been found had all remained in the blood. Assuming that the volume of blood in this rabbit was about 100 cc. 10 mgm. of cholin added to this would have given a concentration of 1 in 10,000; the concentration found was about 1 in 66,000 and this was due in part to the cholin already present.

Experiment March 26, 1915 (fig. 7). Cat ("Tiger") male, 3.07 k. 3 cc. blood drawn from femoral artery. 20 mgm. cholin chloride in 1 cc. Ringer's solution injected rapidly into right saphenous vein; blood-pressure fell from 119 to 67 mm. mercury; heart slowed from 40 to 16 beats in 10 seconds. Blood-pressure and heart rate soon returned to almost normal. One minute after the injection of cholin began to collect 4 cc. blood from left femoral artery; some delay from clotting; blood-pressure now varies between 74 and 106 mm. 11 minutes after the injection of the cholin began to draw 4 cc. blood from the artery; some delay from clotting; blood-pressure now 90 to 104 mm. mercury.

The blood was centrifugalized and the serum treated as usual. The tracings in figure 7 show the following:

1. Comparison of a and c shows that the "1 minute serum" was much more than $2\frac{1}{2}$ times as active as the normal serum.
2. Comparison of b and c shows that the "1 minute serum" was far more active than the "11 minute serum."
3. Comparison of a and b shows that the "11 minute serum" was more active than the normal serum.

Thus there was still some increase in the amount of cholin in the serum 11 minutes after the intravenous injection of 20 mgm. of cholin but evidently much had disappeared. The extent to which the cholin had disappeared in 1 minute is shown by the following:

A comparison (upon another frog heart) between the activity of the "1 minute serum" and acetyl-cholin showed that the "1 minute serum" had an activity corresponding to a little more than 1 part of acetylated cholin 1 in 100,000. Assuming that the cat in this experiment had 150

cc. of blood, 20 mgm. of cholin injected into this would have given a concentration of 1 part in 7,500; instead of this, however, the concentration found was only slightly greater than 1 part in 100,000.

In order to determine whether the cholin which disappears from the serum so rapidly accumulates to any considerable extent in the heart (an organ profoundly affected by cholin) the heart of the rabbit

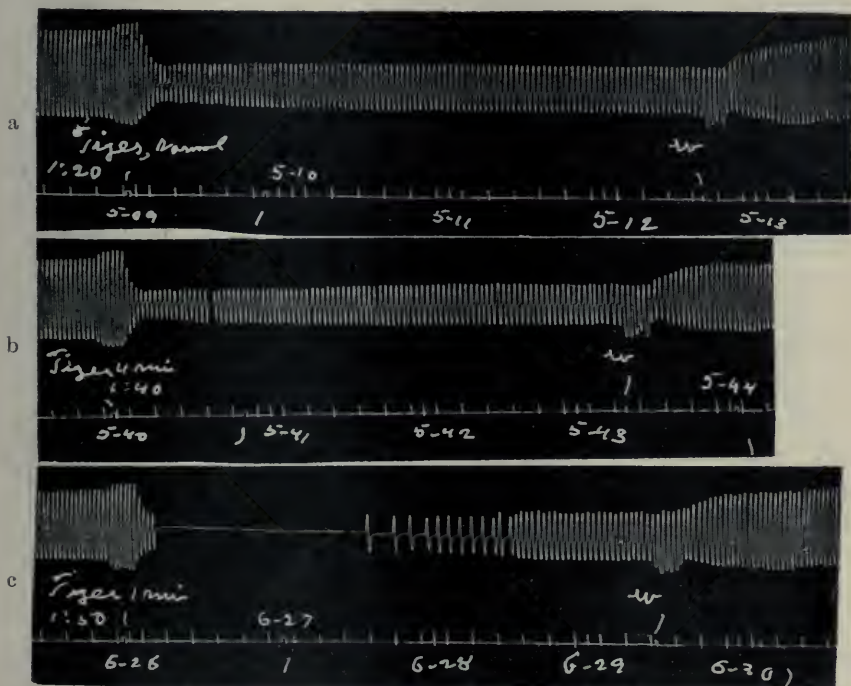


FIG. 7. EXPERIMENT MARCH 26, 1915. FROG'S HEART

The heart was not very sensitive.

a. 5-09. "Tiger, normal 1:20" was the acetyl-cholin, 1 in 20, obtained from 1 cc. of the serum before the cholin injection.

b. 5-40. "Tiger 11 minutes 1:40" was the acetyl-cholin, 1 in 40, obtained from 1 cc. of the serum 11 minutes after the cholin injection.

c. 6-26. "Tiger, 1 minute 1:50" was the acetyl-cholin, 1 in 50, obtained from 1 cc. of the serum 1 minute after the cholin injection.

used in the experiment of February 25 (see above) was removed shortly after the cholin had been injected; 1 gm. of the heart muscle was treated as was the skeletal muscle in the experiment of February 5 (see above)

and the activity of the acetylation product compared with that obtained from 1 gm. of the heart of a normal rabbit. The tracings in figure 8 show that fully as much (probably somewhat more) acetyl-cholin was obtained from the normal heart as from the heart of the rabbit which had received an intravenous injection of 10 mgm. of cholin a short time previously. The amount of cholin in the heart was very small, apparently not much more than 0.005 mgm. in 1 gm. and the fact that 10 mgm. of cholin had been injected into the jugular vein a short time previously had had no appreciable effect.

(This experiment also showed that normal heart muscle had between 2 and 3 times as much cholin as the serum of the animal, that is, there was about the same relation between the cholin content of the heart muscle and the serum of the rabbit as was found between the serum and skeletal muscle of the guinea pig in the experiment of February 5, figure 5.)

Cholin in the blood serum after removal of the suprarenals. Frequently since the discovery that a considerable amount of cholin may be obtained from the suprarenal glands, it has been suggested that these organs are related in some special way to the destruction or metabolism of cholin in the body.¹¹ This suggestion has been tested experimentally and an increase in the cholin content of the blood serum after removal of the suprarenals reported; these results have not (rightly so in my estimation) been found entirely convincing.¹² Loewi and Gettwert have recently reported experiments upon frogs from which they conclude that in these animals after removal of the suprarenals cholin (or a substance having the physiological action of cholin; the substance was not isolated) was present in increased amount in the blood; this was especially marked if the muscles of the frog were stimulated.

I have performed the following experiments upon this subject.

Experiment January 27, 1915. The suprarenals of a female cat, 2.5 k, were removed (posterior route) at 2.30 in the afternoon; good recovery

¹¹ This thought occurred to me soon after I found cholin in the suprarenal glands and some experiments, yielding negative or inconclusive results, were performed in 1906 to determine whether there is an increase in the cholin of the blood serum after removal of the suprarenals.

¹² The literature on this subject has been reviewed by Loewi and Gettwert, Pflüger's Archiv, 1914, 158, p. 29.

from the operation; animal seemed normal at 6 p.m. Was alive but lying down at 9 a.m. the next morning; was dead 30 minutes later (19 hours after the operation). Blood was drawn from the heart, the auricles of which were beating feebly; the blood was treated in the usual way and the activity of the acetylation product compared with that of

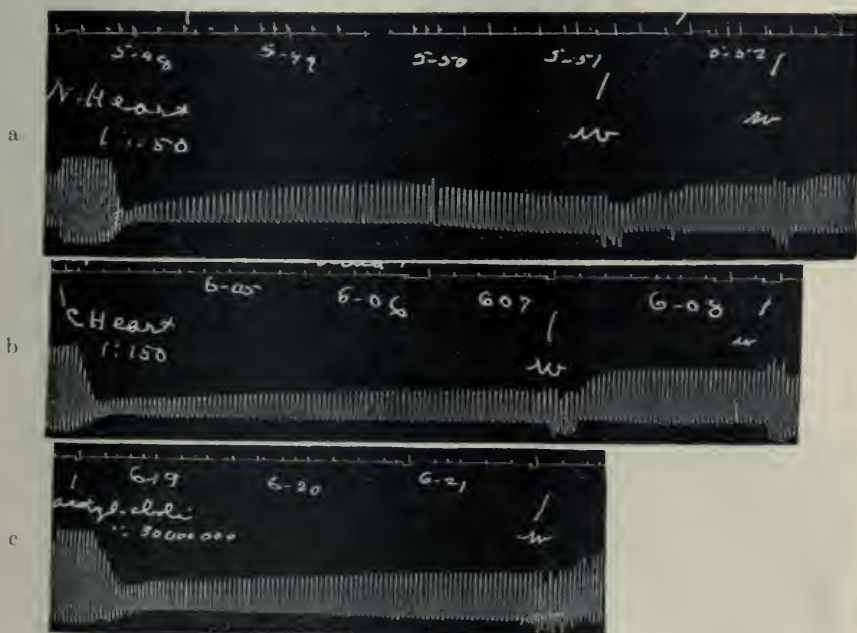


FIG. 8. EXPERIMENT FEBRUARY 25, 1915

(See previous protocol; the frog heart from which these tracings were obtained was not that giving the tracings in figure 6.)

a. 5-48. "N Heart 1:150" was the acetyl-cholin, 1 in 150, obtained from 1 gm. of a normal heart.

b. 6-05. "C Heart 1:150" was the acetyl-cholin 1 in 150 obtained from 1 gm. of the heart of the rabbit into which cholin had been injected.

c. 6-19. Acetyl-cholin 1 in 30,000,000.

blood drawn before the removal of the suprarenals and with that of acetylated cholin.

The tracings in figure 2 (b and c, also d and c) showed that the serum after removal of the suprarenals was more active than before the operation; other comparisons indicated that the former was 25 per cent to 30 per cent more active. The untreated serum, in dilutions 1 in 10,

caused an increased amplitude of beat, the normal serum being slightly more active; undiluted the serum after operation caused a marked diminution of the amplitude, followed, when fresh Ringer's solution was employed, by a marked stimulation.

Experiment March 26, 1915. (1) Gray cat, female, 3.22 k. All vessels to and from the suprarenals ligated at 3.15 p.m. Animal soon recovered from the operation; the next day it was up but sluggish. At 2.30 p.m. (about 23 hours after the operation) ether was given; some excitement under ether. A cannula was inserted into the carotid but the cat died before the blood-pressure could be taken. Blood removed from the heart and centrifugalized; it was noted that the bulk of the corpuscles was greater and the amount of serum obtained was less than in the case of the blood from the two following cats although the blood of all was centrifugalized for the same time and at the same rate. The serum was slightly pink. (2) Slate colored cat, female, 2.06 k. Suprarenals tied off at 4.30 p.m.; soon recovered. Up but sluggish next day; hair rough. At 3.50 p.m. (23½ hours after the operation) etherized and blood-pressure experiment performed; the blood-pressure varied between 50 and 70 mm. mercury; it was not affected by atropine. (This speaks against the low blood-pressure being due to cholin.) 7 cc. of blood drawn from carotid (before the administration of atropine) and centrifugalized; the serum was slightly pink but less so than that of "Gray" above. (3) "Tiger-faced" cat, female, 3.07 k. Blood from femoral artery; centrifugalized.

One cubic centimeter each of the three specimens of serum obtained from the above cats ("Gray", "Slate" and "Tiger") were treated in the usual manner and the activity of various dilutions of the acetylation products compared with each other. Tracings illustrating their relative activity are shown in figure 9 and figure 7; these show that

(1) The activity of the serum of "Slate" (adrenalectomized) and of "Tiger" (normal) was about equal (comparison of a, b, f of figure 9 and a of figure 7).

(2) The activity of "Gray" (adrenalectomized) was more than twice as great as that of "Tiger" or of "Slate" (comparisons of c, e and f).

Other comparisons showed that the activity of "Gray" was about three times as great as that of "Tiger."

Thus in the blood of one of the adrenalectomized cats there was no, or at most only a very slight, increase in the amount of cholin; in the other there was apparently a marked (three-fold) increase. Even in the latter, however, the absolute quantities of cholin involved were

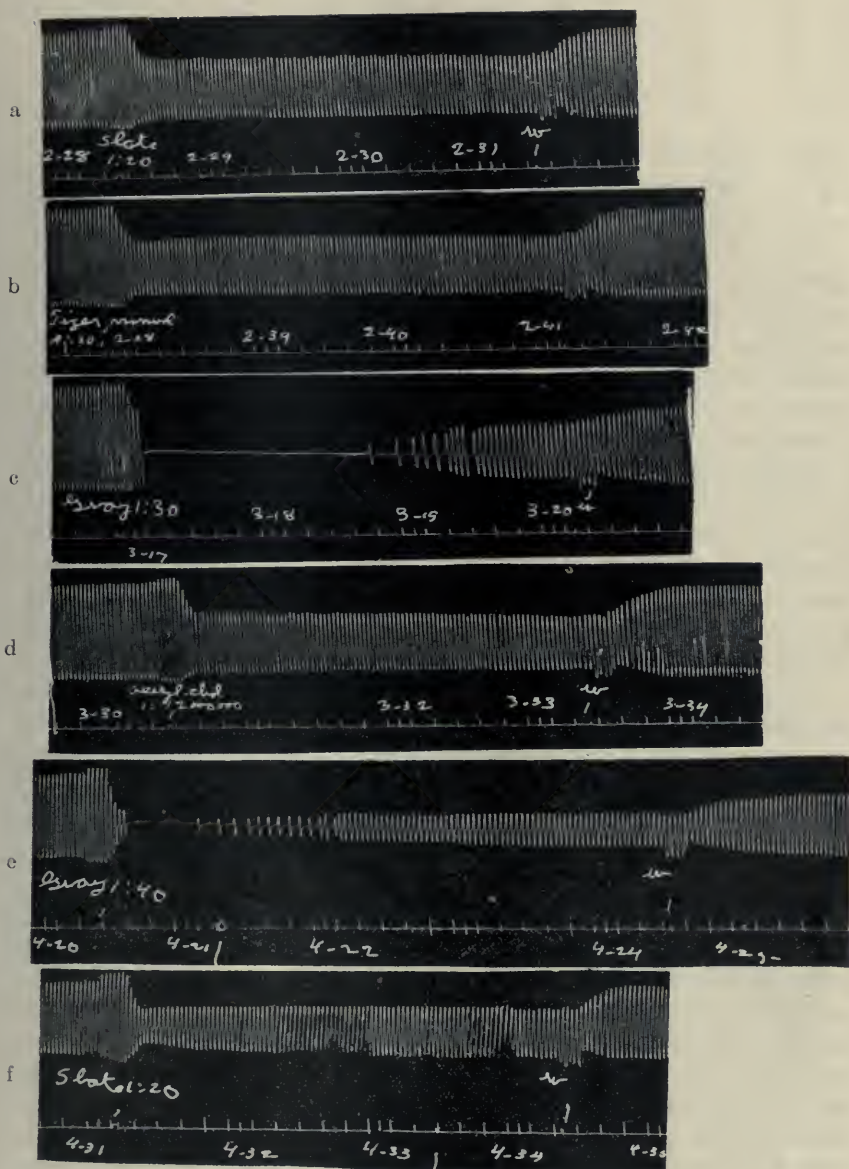


FIG. 9. EXPERIMENT MARCH 26, 1915. FROG'S HEART

- a. 2-28. "Slate 1:20" was the acetylation product obtained from 1 cc. of the serum of an adrenalectomized cat; dilution 1 in 20.
- b. 2-38. "Tiger, normal, 1:30" was the acetylation product obtained from 1 cc. of the serum of a normal cat; dilution 1 in 30.
- c. 3-17. "Gray, 1:30" was the acetylation product obtained from 1 cc. of the serum of an adrenalectomized cat; dilution 1 in 30.
- d. 3-30. Acetyl-cholin, 1 in 12,000,000.
- e. 4-20. "Gray 1:40" same as c; dilution 1 in 40.
- f. 4-31. "Slate 1:20" same as a.

(The tracings of figure 7 were taken soon after the above and may be used for comparison.)

very small: there was scarcely more than 1 mgm. in 125 cc. of serum or, since the amount of blood in the cat (which weighed 3220 gm.) may be estimated as about 160 cc. there was less than 1.3 mgm. of cholin in the entire blood, an amount to which, apparently, not much significance could be attached. There is, however, another way of looking at this subject: As was shown above (see fig. 7) 20 mg. of cholin was injected intravenously into the normal cat ("Tiger"), an amount sufficient to give a concentration in the blood of 1 in 7500 but blood drawn from the femoral one minute later showed a concentration of only about 1 in 100,000 and this was due in part to the cholin originally present in the serum. Comparison of tracing c (fig. 9) and of c of figure 7 indicate that the serum of the adrenalectomized cat (Gray) had nearly as much cholin as had that drawn 1 minute after the injection of the 20 mg. of cholin into the normal cat. Hence the presence in the blood of only a slightly abnormal amount of cholin does not show that comparatively large amounts have not been or are not being added to the blood.

If, by the use of methods more accurate than those now available, an increased amount of cholin is clearly shown to be present in the blood after removal of the suprarenals (or in other pathological conditions) the question will arise whether this has resulted from an increased formation of cholin or from a retention of that normally being formed; nothing is known concerning the latter question.¹³

Attention was called above to the unusually large volume of corpuscles and the small amount of serum which was obtained from the adrenalectomized cat (Gray) which had the larger amount of cholin in the serum. This suggests the possibility that the serum was more concentrated (and that the cholin had not passed from the vessels with the water). Donath¹⁴ has recently found that after removal of the suprarenals there is a concentration of the blood attributable to the increased permeability of the vessels resulting from the loss of epinephrin and Gradinescu¹⁵ found an increase in the number of red blood corpuscles after removal of the suprarenals.

¹³ A similar question arose in regard to the presence of thyroid secretion in the blood of Grave's disease: I obtained a reaction which seemed to indicate the presence (in increased amount) of such secretion but was unable to obtain this reaction in the blood of animals to which very large doses of thyroid had been fed; I suggested that perhaps in Grave's disease there is a question of diminished destruction or excretion of thyroid secretion as well as a question of increased formation (Jour. Amer. Med. Assoc., 1907, 49, 240).

¹⁴ Donath: Arch. f. exper. Path. u. Pharm., 1914, 77, p. 1.

¹⁵ Gradinescu, Pfüger's Archiv., 1913, 152, 187.

In the following experiment the serum of three rabbits was compared: (1) normal (2) rabbit from which the kidneys had been removed (3) rabbit from which the suprarenals were removed.

Experiment March 19, 1915. (1) Normal rabbit, male, 1.7 k.; blood was drawn from the carotid. (2) Rabbit, male, 1.78k. Kidneys had been removed 48 hours previously; no symptoms except slight twitchings. Etherized and record taken of blood-pressure; latter fell in course of 10 minutes from 59 mm. to zero. Blood from heart immediately after death. The serum was almost colorless but opalescent. (3) Rabbit, male, 1.78 k. Suprarenals removed 9.30 a.m.; did not recover from ether and the operation beyond ability to hold up head; died in $3\frac{1}{2}$ hours. Blood removed from the heart and great veins; the serum was bright pink. (The splanchnic nerves were probably severely injured during the operation. The suprarenals were removed from another rabbit by the same technique while a record of the blood-pressure was being taken; the blood-pressure fell at once from 62 mm. to 40 mm. and continued to fall slowly until death occurred about an hour later.)

The serum of the blood obtained from the above animals was tested in the usual way and the activity of their acetylation products compared. The tracings in figure 10 show:

(1) Comparison of b, c and d shows that the acetylated serum of the rabbit from which the suprarenals had been removed was about 6 times as active as the normal serum.

(2) Comparison of e and f shows that the serum of the nephrectomized rabbit was more than 3 times as active as that of the normal rabbit. (Other comparisons showed the former to be 5 times as active as the latter.)

The serum of the rabbit from which the suprarenals had been removed thus contained many times as much cholin as that of the normal rabbit. But since a similar difference was found between the serum of the normal and the nephrectomized animals it seems probable that the result in the former case does not indicate any specific relation of the suprarenals to the amount of cholin in the blood.

The untreated serums, in dilutions 1 in 10, in all 3 cases caused a marked stimulation of the frog's heart when this was beating with diminished amplitude after long experimentation; no differences were noted in the action of the three serums.

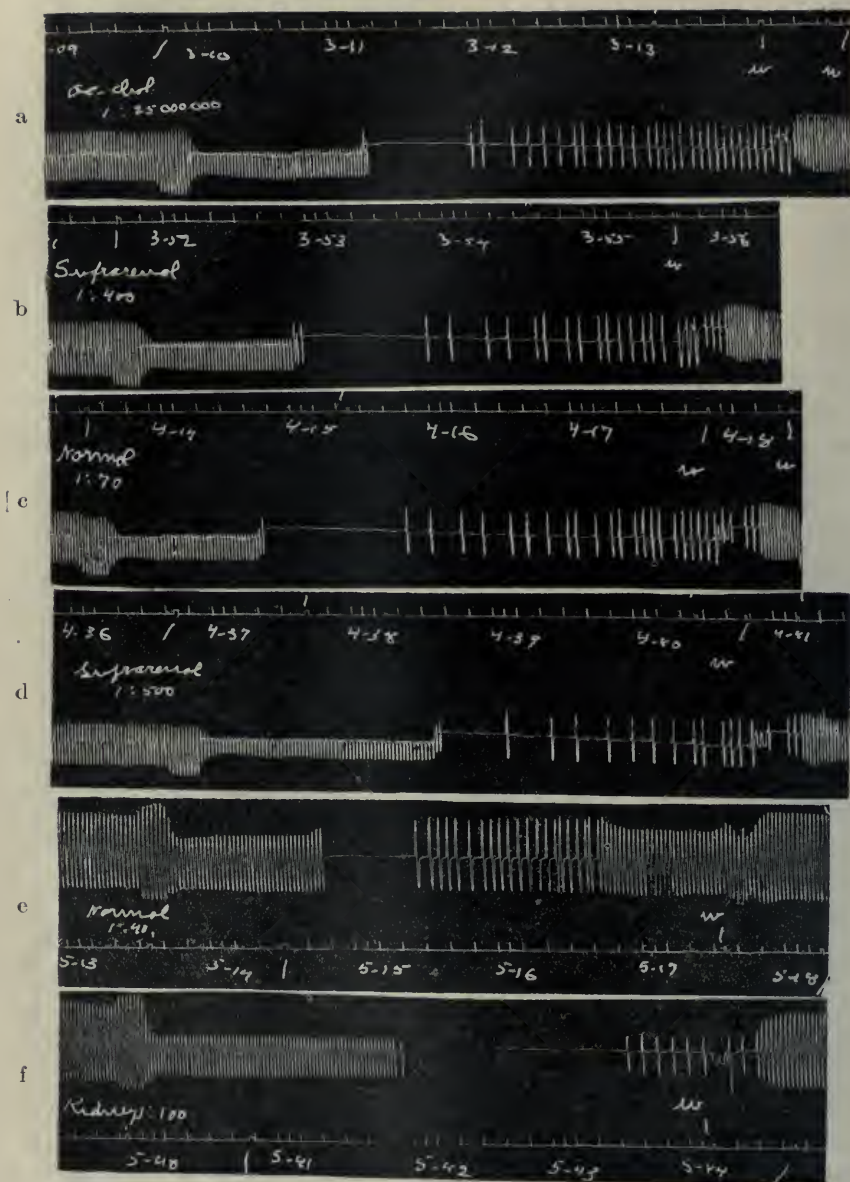


FIG. 10. EXPERIMENT MARCH 19, 1915. FROG'S HEART

- 1 heart was used for a, b, c and d; another (less sensitive) for e and f.
- 3-10. Acetyl-cholin 1 in 25,000,000.
 - 3-52. "Suprarenal 1:400" was the acetyl-cholin, 1 in 400, obtained from 1 cc. of the serum of the rabbit from which the suprarenals had been removed.
 - 4-14. "Normal 1:70" was the acetyl-cholin, 1 in 70, obtained from 1 cc. of normal rabbit serum.
 - 4-36. "Suprarenal 1:500" same as c; dilution 1 in 500.
 - 5-13. Same as c but dilution of 1 in 40.
 - 5-40. "Kidney 1:100" was the acetyl-cholin, 1 in 100, obtained from 1 cc. of the serum of the nephrectomized rabbit.

Attention may be called here to figure 5 (experiment February 5) which shows that after removal of the suprarenals (guinea-pig) there was no increase of cholin in the skeletal muscle; other tracings in this experiment show the same. Attempts were also made in this

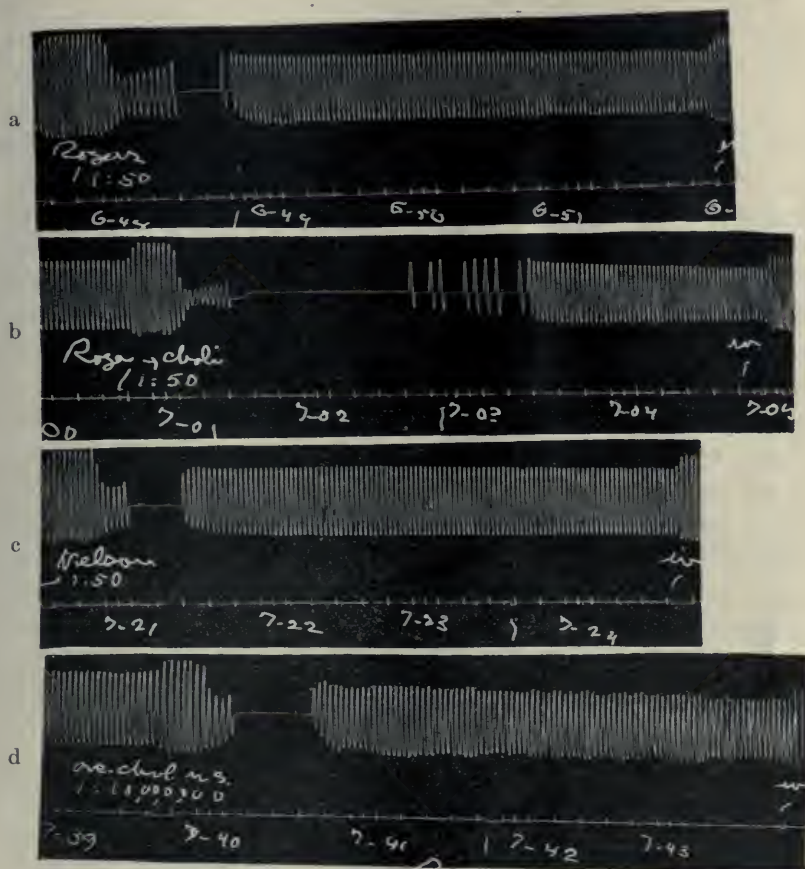


FIG. 11. EXPERIMENT MARCH 3, 1915. FROG'S HEART

a. 6-48. "Rogers 1:50" was the acetyl-cholin 1 in 50, obtained from 1 cc. of the serum of a normal man.

b. 7-01. "Rogers and cholin 1:50" was acetyl-cholin 1 in 10,000,000 plus the acetyl-cholin, 1 in 50, obtained from a.

c. 7-21. "Nelson, 1:50" was the acetyl-cholin, 1 in 50, obtained from 1 cc. of the serum of a case with nephrectomy with non-coagulable N retention.

d. 7-40. Acetyl-cholin 1 in 10,000,000.

experiment (on the guinea pig) to compare the cholin content of the urine of the adrenalectomized animal with that of a normal guinea pig. The urine of the former, however, was obviously contaminated with a discharge from the genital glands which, as is well known, contains cholin. Even with this discharge, however, the urine contained cholin equivalent to only about 1 in 75,000; the cholin content of the urine of the normal guinea pig was about 1 in 110,000.

Cholin in the blood of man in certain pathological conditions.

(a) Nephrectomy; non-coagulable N retention. As was noted above relatively much more cholin was found in the serum of a rabbit from which the kidneys had been removed than in that of a normal rabbit; the absolute amount present, however, was very small. In the experiment the tracings of which are shown in figure 11 the activity of the acetyl-cholin obtained from 1 cc. of the serum of a case of nephrectomy (non-coagulable N = 150+ mgm. per 100 cc.)¹⁶ was compared with that obtained from a normal adult and with that of a solution of acetyl-cholin of known strength. The results show that the amount of cholin in the two specimens of serum was the same; that is, the nitrogen of cholin had not contributed to the increase of non-coagulable N in the case of nephrectomy. (The tracings also show that the cholin content of both the normal and abnormal serum was slightly less than 1 mgm. in 200 cc.; and that 0.0001 mgm. of cholin added to 1 cc. of serum was fully recovered.)

(b.) *Dementia precox. General paresis. Alcoholic hallucinosis* (with bradycardia).¹⁷

¹⁶ I am indebted to Dr. W. W. Palmer of the Massachusetts General Hospital for these samples of blood and for the statement as to the non-coagulable N.

¹⁷ The serum in these cases was obtained from the Psychopathic Hospital through the kindness of Drs. Southard and Adler. I am indebted to Drs. Solomon and Grabfield for the following notes on the cases:

Case 1. Dementia precox. S, age 23. History: gives nothing of importance except statement that he had syphilis. Present attack began suddenly day before entrance. On entrance was depressed, speech slow and difficult, memory somewhat clouded, disoriented, delusions of persecution, cerea flexibilitas shown. Except for the cerea flexibilitas the physical examination was negative. Blood serum negative to Wassermann reaction. Pulse rate between 80 and 90 per minute.

Case 2. General paresis. F, age 29. History: nothing very definite in way

One cubic centimeter serum from one case of each of these conditions was treated, in the usual way, for cholin. The result are shown in figure 12 from which it appears that the serum of the case of general paresis contained somewhat more cholin than did that of the case of alcoholic hallucinosis and also more than did the case of dementia precox but the differences were not striking.

No comparisons were made between the activity of these serums and that of normal human serum but comparisons between the activity of the serum of the case of alcoholic hallucinosis and that of a solution of acetylated cholin of known strength showed that the former had a cholin content corresponding to about 1 mgm. in 250 cc.—a content of the same order or perhaps a little less than that found in specimens of normal human serum.¹⁸ The serum itself (that is untreated) had no effect on the frog heart.

c. *Thyroid disease.* The blood serum in 2 cases of thyroid disease was examined;¹⁹ (1) a woman of 65 suffering from post-

of history obtainable. He tells of a period of depression lasting three months, nine months before admission, followed by a remission. Present attack started only a few days before admission. Is very excited, boisterous, and expansive. There is great psycho-motor activity. Some disorientation, but memory on the whole seems intact. Admits chancre 10 years ago.

P. E. Shows a thin, somewhat emaciated man. There is slight axillary and inguinal adenopathy. Pupils are irregular and react within narrow limits to light, better to accommodation. Knee and ankle jerks are very lively. No abnormal reflexes elicited. No tremors or speech defect. No Romberg, no sensory disturbance. Wassermann reaction: serum and spinal fluid positive. Spinal fluid shows abnormally large amounts of albumen as precipitated by trichloroacetic acid (Mestrezat test) and globulin as precipitated by half saturation of ammonium sulphate (Nonne-Apelt test). Cells 127 per cm. Gold sol test positive for paresis.

Case 3. Alcoholic hallucinosis. F, age 46. Past history not reliable. Present mental condition probably of but several weeks duration. History of alcoholism. Auditory, visual and tactile hallucinations, ideas of jealousy against wife based on auditory hallucinations. Orientation intact. Remote memory poor, recent memory good. At times depressed on account of his hallucinations.

Bradycardia, pulse rate around 40 per minute. Systolic pressure 120, diastolic 80. Physical examination otherwise negative. Wassermann reaction negative in blood serum and fluid.

¹⁸ See figure 11. Another specimen of normal human (negro) serum was found to contain about 1 mgm. of cholin in 250 cc.

¹⁹ I am indebted to Dr. Goetsch of the Peter Bent Brigham Hospital (service of Dr. Cushing) for these specimens.

operative myxedema; (2) a man of 39 with a circumscribed adenoma of the thyroid. The cholin content in the serum of these two cases was about the same; it was distinctly less than that of any of the human serums the examination of which is

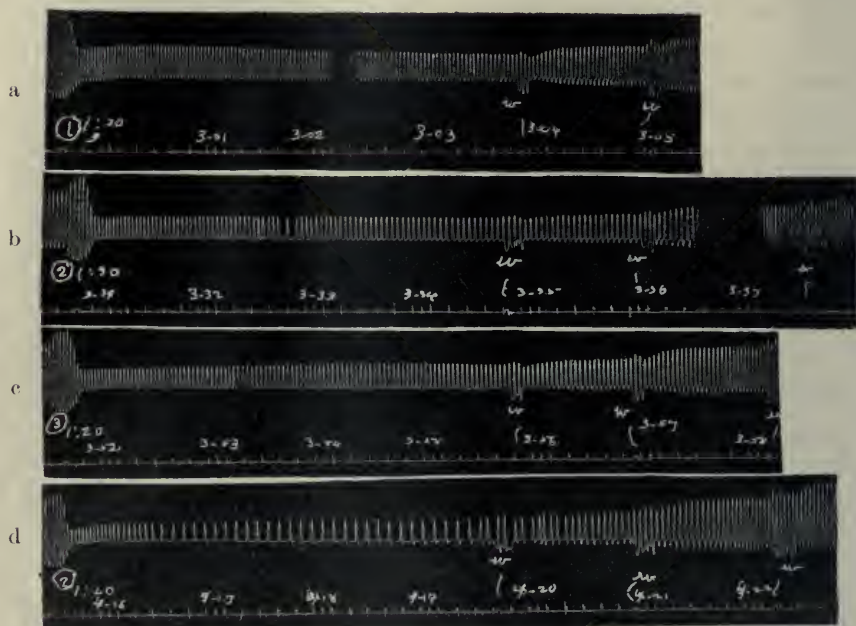


FIG. 12. EXPERIMENT FEBRUARY 20, 1915. FROG'S HEART

"w" indicates that the acetyl-cholin solution was replaced by fresh Ringer's.

a. 3. "(1) 1:30" was the acetyl-cholin, 1 in 30, obtained from 1 cc. of the serum of a case of dementia precox.

b. 3-34. "(2) 1:30" was the acetyl-cholin, 1 in 30, from 1 cc. of the serum of a case of general paresis.

c. 3-52. "(3) 1:20" acetyl-cholin, 1 in 20, from 1 cc. of serum of case of alcoholic hallucinosis.

d. 4-16. "(2) 1:20" same as b; dilution 1 in 20.

reported above but probably no significance is to be attached to this in view of the very small amounts of cholin involved (from 0.002 to 0.005 mgm. per cubic centimeter).

*Cholin in the cerebro-spinal fluid.*²⁰ The method was applied to only a few specimens of cerebro-spinal fluid and these did not include cases in which, according to the well known views of Mott and Halliburton, cholin would be most probably found. The specimens of cerebrospinal fluid (which, in all but one case, were obtained by lumbar puncture)²¹ were treated in the same way as were the specimens of blood serum in the experiments discussed above.

The cases studied and the amounts of cholin found were as follows:

- (1) T; chronic meningitis (?); female; fluid yellow. 1 mgm. cholin in 1000 cc.
- (2) F; alcoholic hallucinosis. 1 mgm. cholin in 2000 cc.
- (3) Congenital hydrocephalus; child 7 months; fluid from ventricle. 1 mgm. cholin in 2500 cc.
- (4) McD; tumor of cerebellum; man aged 33; fluid (which was drawn 3 weeks after operation) yellow. 1 mgm. cholin in 2500 cc.
- (5) L.; syringomyelia. 1 mgm. in 3000 cc.
- (6) B; trigeminal neuralgia? 1 mgm. in 3000 cc.
- (7) F; fracture of skull; fluid drawn 1 week after injury. 1 mgm. in 4000 cc.

The above figures are only approximations which is readily understood when it is remembered that the actual amounts of

²⁰ Inasmuch as the examinations here reported were made entirely by the method outlined above (which is new) and the results are not especially striking, I shall not discuss the extensive and controversial literature on the subject of the occurrence of cholin in the cerebro-spinal fluid (See Halliburton: *Ergebnisse der Physiologie*, 4, 23, 1905; Webster: *Biochem. Jour.*, 4, 117, 1909; v. Fürth: *Probleme d. physiolog. u. patholog. chemie*, 1, p. 190; etc.). For the same reasons I omitted a discussion of the literature on the occurrence of cholin in the blood serum. Moreover the methods hitherto employed do not preclude the formation of cholin, as the result of chemical and other manipulations, from lecithin; I was interested primarily in the occurrence of free cholin or of very simple derivatives of it, having the same solubilities as cholin, and to the presence of which physiological or pathological interest may attach. As has already been stated it is possible that the small amounts of cholin which I found were really derived from lecithin in the course of extraction.

²¹ I am indebted to Drs. Harvey Cushing and Towne of the Peter Bent Brigham Hospital for all of these specimens, and the notes; with the exception of No. 2; this was from the case described in note 17.

cholin involved were only 0.00025 to 0.001 mgm. (the determinations being made upon 1 cc. of fluid in each case). The differences in activity of the different specimens were, however, very striking: thus the acetyl-cholin obtained from No. 1, in a dilution of 1 in 10 (corresponding to a solution of acetyl-cholin 1 in 10,000,000) brought the frog heart to a standstill which continued for four minutes although the solution in the heart was replaced after one minute by fresh Ringer's solution and this was repeated several times before the heart began to beat; No. 4, in a dilution of 1 in 10, brought the heart to a standstill of 70 seconds whereas No. 5, in a similar dilution, had no effect upon the rate of the heart but diminished the amplitude slightly. The results also show that the cholin content of the cerebro-spinal fluid is much lower than that of any of the samples of blood serum I examined; the latter had from 5 to 20 times as much. The only instances in which the cholin content of the blood serum and cerebro-spinal fluid of the same case was compared was No. 2 (alcoholic hallucinosis); the serum contained about 8 times as much cholin as did the cerebro-spinal fluid.

Cholin in saliva. Houdas²² reported the presence in the saliva of horses of a substance, or substances, giving certain micro-chemical tests similar to those given by cholin; no attempt at a quantitative estimation was made. I performed the following experiment: 1 cc. of human saliva was treated in the manner described for blood serum; the acetylation product in a dilution of 1 in 10, was slightly less active than a dilution of acetylated cholin, 1 in 20,000,000. That is, the saliva contained slightly less cholin than a solution of cholin, 1 in 2,000,000; in other words the cholin content of the saliva was of the same order as that of the cerebro-spinal fluid and many times less than that of the blood serum.

Cholin in the amniotic fluid. One cubic centimeter of the amniotic fluid of a cat (the embryos were about 25 mm. long) was treated with acetone, etc. in the manner described for blood serum. The acetylation product corresponded in activity to

²² Houdas: Compt. rend. acad. d. sciences, 1913, 156, 824.

that of a solution of acetylated cholin, 1 in 260,000; that is, the cholin content of the amniotic fluid of the cat was of the same order as that of the blood serum of the cat and much greater than that of (human) cerebro-spinal fluid or (human) saliva.

DISCUSSION

In none of the above experimental or pathological conditions was a marked increase in the amount of cholin in the blood serum or cerebro-spinal fluid found. It would be unwise, however, to conclude that even a slight increase may be without significance. In view of the fact, brought out above, that over 90 per cent of the cholin injected intravenously may leave the blood within one minute, even a small increase in the amount present at one time may indicate a very greatly increased production and this might have important physiological effects. And, as a matter of fact, the amount actually found in some of the animals after removal of the suprarenals was greater than that which would have immediately resulted from the injection of an amount (1 mgm. per kilo for example) which ordinarily does cause a fall of blood-pressure. While these experiments clearly show that there is no great accumulation of cholin in the blood serum or cerebro-spinal fluid in the experimental and pathological conditions examined they do not answer the question, however, whether there may not be an increased production of cholin and whether cholin after all may not be a factor in the causation of certain symptoms.²³ At present, however, we have no grounds for believing that cholin does have any significance in physiological or pathological processes, however attractive such a hypothesis may be.

From my first publications on the subject of cholin, I have emphasized the point of view that if cholin is involved in any way in pathological or physiological processes it is very probable that it is cholin in the form of some much more active com-

²³ For the same reasons it may be questioned whether the elaborate studies which have been made on the occurrence of epinephrin in the blood have thrown any real light on the question of a possible relation between the activity of the suprarenal glands and conditions of hypertension.

pound. There is some evidence that such compounds exist or may be formed in the body or from substances present in the body. Thus I²⁴ found evidence of the existence in the suprarenal glands of a substance having about fifty times the physiological activity of cholin and which readily yielded cholin as the result of simple chemical manipulations. Taveau and I and Menge²⁵ prepared a large number of cholin derivatives, much more active than cholin itself, which might conceivably be formed in the body. Very interesting in this connection are the recent studies of Delezenne and Fourneau²⁶ on "lysocithine" a cholin ester formed from lecithin, and other cholin esters which are extremely active hemolytic agents.

No evidence, however, was obtained in the foregoing experiments of the occurrence of any very active compounds of cholin;²⁷ in none of the cases did the blood serum itself (that is without acetylation) give results differing from those obtained with normal serum.

SUMMARY

(1) A physiological test is described by which 0.00001 mgm., and probably less, cholin may be detected.

(2) Although an increased amount of cholin was found in the blood serum of some (but not of all) animals after removal of the suprarenal glands, it is doubtful if this indicates any specific relation between these glands and the metabolism of cholin.

²⁴ Hunt: Amer. Jour. Physiol., 1901, 5, p. vi.

²⁵ Menge: Jour. Biol. Chem., 1911, 10, 399; 1912, 13, 97; Bull. 96 U. S. Hygienic Laboratory 1914; Hunt, Jour. of Pharm. and exp. Therap., 6, 477, 1915; cf. Dale: *ibid*, 6, 147, 1914.

²⁶ Delezenne and Fourneau, Bull. soc. chim., 4, 15, 421, 1914.

²⁷ Except possibly in the fact that in certain of the cases where the blood showed a relatively high cholin content some hemolysis had occurred; possibly a compound of cholin was present which was very hemolytic and which served as the source of the cholin. I do not think that the hemolyzed blood corpuscles was the source of the increased amount of cholin for no more was obtained from serum to which 10 per cent of a solution of red corpuscles dissolved by distilled water had been added than from a correspondingly greater amount of serum alone. This subject, however, is being investigated further.

(3) There was no increase of cholin in the striated muscle after removal of the suprarenals (one experiment).

(4) No marked increase of cholin in the blood serum or cerebro-spinal fluid was found in a number of pathological conditions.

(5) The cholin content of the cerebro-spinal fluid and of the saliva (one experiment) was much less; that of the aminotic fluid (one experiment) about equal to and that of the urine and cardiac and skeletal muscle somewhat greater than that of blood serum.

(6) Cholin injected intravenously disappeared from the blood with great rapidity; there was not an increased amount in the heart.

ACTION OF THE OPIUM ALKALOIDS, INDIVIDUALLY AND IN COMBINATION WITH EACH OTHER, ON THE RESPIRATION¹

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The effect of opium on the respiration is next to its narcotic and pain-relieving action, therapeutically perhaps the most important, and toxicologically certainly the most striking. Any one seeing a case of morphin or opium poisoning cannot fail to be impressed by the extreme slowness and shallowness of the patient's breathing, which is a dominant feature of the clinical picture. It is therefore not at all surprising to find more or less attention devoted to the respiratory phenomena in all the earlier physiological and pharmacological literature on opium. Most of the earlier investigators in this field, however, have confined their studies to the action of morphin, and later investigators to the derivatives of morphin, heroin, dionin, etc. Concerning the behavior of the other primary opium alkaloids we find hardly any mention, with the exception of the classical work of v. Schröder (1) who, however, unfortunately treated of them only superficially. Inasmuch as I have already in a preceding paper shown that the rarer opium alkaloids exert quite an important and interesting pharmacological action on the coronary circulation (2), and also exhibit a remarkable behavior when combined with each other, it was desirable and important to ascertain their influence on respiration. Moreover, even in the more intensive comparative studies of morphin and its derivatives, such as those by Dreser (3), Impens (4), Mayor (5), and others, the respiratory phenomena are considered almost entirely

¹ This investigation has been endowed by a grant from the American Medical Association through the Council on Pharmacy and Chemistry.

from two points of view, namely, the effect on the *rate* of respiration, and the effect on the *volume* of air expired in a given time. Such data in the light of recent physiological work are very inadequate. A comprehensive study of the pharmacological action of a drug on the respiration is not such a simple matter; indeed it is much more difficult than the study of circulation.

The various ways in which a drug may affect the respiration may be conveniently summed up as follows:

1. It may stimulate or depress the respiratory center.
2. It may affect the respiration indirectly through increasing or decreasing the metabolism.
3. It may affect the respiration indirectly through the circulation, namely through circulatory changes in the brain and spinal cord, through peripheral vasodilation and consequent loss of temperature, etc.
4. It may alter the general character of the breathing by making it rapid or shallow, slow or deep, thoracic or abdominal, through changes in the mechanical conditions within the chest or abdomen, as, for instance, by acting on the various viscera or on the thoracic or abdominal musculatures.

5. It may alter respiration by acting on the broncho-constrictors or broncho-dilators and in that way altering the so-called "dead space" which necessitates a compensatory change in ventilation.

In order to study properly, therefore, the action of a drug on the respiration from the therapeutic point of view, we must not only study the (1) *rate* of respiration, and the volume of air respired or (2) *total ventilation*, but also the carbon dioxide tension, which enables us to determine the (3) *alveolar* or *true ventilation* of the lungs, and again the effect on the (4) *respiratory center*, and lastly the (5) action on the *bronchi*. We must furthermore constantly bear in mind the effect of the drug (6) on the *circulation*, especially in regard to the vital centers. It is well to call particular attention at this point to the fact which has been overlooked by earlier workers on the subject that it is the *alveolar* and not the *total ventilation* which is of importance. The total ventilation or the amount of air respired by an animal per minute, does not convey an accurate conception of the true aëration of the blood, as it does not differentiate between the amount of air

metabolized by the alveoli and the air in the dead space. Yet, as Edsall and Means (6) well put it, "the chief function of the respiratory center is to maintain a ventilation of the lung alveoli sufficient to keep the tension of carbon dioxide at a definite level." It is, therefore, the alveolar ventilation and the carbon dioxide tension rather than the total respiration and respiration rate which are of chief importance in studying the condition of the respiratory center.

In the present research an attempt has been made to study somewhat intensively along the above lines of inquiry the effect of some of the principal opium alkaloids on the respiration individually and in combination with each other.

OLDER METHODS OF STUDY

The various methods of studying respiration heretofore employed, have for their object primarily the study of the rate and total volume of respiration and may be divided broadly into three classes. In the first place there is the simple device of registering the respiratory movements of the chest through some sort of a stethograph or pneumograph. This of course is only an indirect method, and subject to great variations, depending on the condition of the respiratory muscles, the movements of the viscera, etc. Although inadequate it is simple and convenient and for that reason has been and is largely employed in kymographic work.

Another method of studying respiration is by measuring the aëration of the lungs directly through a tube inserted in the trachea. The chief objection to this method is that the tracheal tube, however carefully inserted, is a constant irritant to the animal: in fact it is a foreign body and acts as such. As a consequence the respiratory rate and depth are greatly distorted, and are not at all indicative of the behavior of the respiration in an intact animal. I have made comparative experiments, in both tracheatomized and non-tracheatomized animals, and have found such enormous differences in the results that I regard this method as entirely unreliable for pharmacological research. The same remarks apply to the method employing a tube or nozzle inserted into one of the nostrils.

A third method of studying the respiration is based on the principle of the body plethysmograph. An ingenuous and improved form of this kind of apparatus has been recently introduced by Cushny (7). In considering critically the merits and defects of this form of apparatus, we note again that it affords only an indirect method of recording the respiration, namely through the movements of the chest and the abdomen, and is therefore also not completely satisfying.

A most important disturbing factor to be traced in almost all the work done on the pharmacology of respiration is the use of anesthetics. It seems hardly necessary to state that anesthesia exerts a profound influence on the respiration of an animal, and for the study of the pharmacological action of another drug, it is absolutely necessary to avoid error from this source if we would obtain any useful data for therapeutic purposes. Strange to say, however, by far the greatest amount of work done in this field has been on anesthetized animals.

METHODS EMPLOYED BY THE AUTHOR

In the present research some form or other of all the above described methods of studying the respiration were employed, on various animals. Thus I have studied the respiration by direct observation without the aid of any recording apparatus in frogs, fish and mice; the stethographic or pneumographic method was employed in operative work on rabbits, cats, and dogs; a body plethysmograph was used in studying the breathing of guinea pigs. All these observations, however, were only of value in obtaining more or less empirical and qualitative data. For more careful and quantitative work it was necessary to devise a special form of apparatus, which I deem it advisable to describe here in detail. The recording tank and the idea of using a breathing mask have been taken from the valuable work of Impens (8) but the rest of the apparatus, has been devised in this laboratory and for the elaboration and construction of the finer parts I am especially indebted to Dr. B. Turner.

DESCRIPTION OF APPARATUS

A rabbit is tied down securely on its back to a rabbit board, *A*. To its head is then adjusted a specially constructed glass helmet, *I*. The rim of the helmet is roughly trapezoidal and not circular in shape, corresponding approximately to the cross-section of a rabbit's head, and is provided with a rubber cushion to fit snugly to the animal's head. From the rim of the helmet downward extends a thick rubber sleeve, made out of a heavy surgical glove, which is drawn over the animal's head, to render the adjustment of the helmet air tight, and this can be further secured by tying the helmet down behind the occiput with a piece of gauze. The helmet pressing on the bony structure does not interfere at all with the breathing or the circulation of the head as may be noted by observing the mucous membranes through the glass. The neck of the helmet, which is made as wide as possible, about 1 to 1½ cm. in diameter, is joined by means of rubber tubing to a wide glass T-tube, *J*, which is connected on the one end with the glass bottle, *G*, and the other end with the gasometer, *B*. The flask *G* is a water valve consisting of a long glass tube *H*, which extends to nearly its bottom and dips just below a layer of water, and a short tube which extends just through the cork and is connected with the T-tube *J*. The gasometer *B*, consists of a large flat tin or galvanized iron tank which is made water tight and has only two openings, through one of which the glass tube *K*, and through the other the tube *D* pass. The tank is filled with water through *K* until the water begins to overflow from the end *E* of the out-flow-tube *D*. The level of *E* is therefore on a level with the surface of the water in the tank. The end *K'* of the glass tube *K* extends *just below* the surface of the water. *M* is a thin glass stopper which fits into a small aperture bored through the cork, and which is taken out when water is being run into the tank through *K*, in order to provide an escape-hole for the air. When *M* is replaced and the end of the tube *K* is shut off with the stopper *L*, the gas tank is ready for registration. With each inspiration the animal draws air in through the tube *H*, into *J*, and thence into the helmet. On

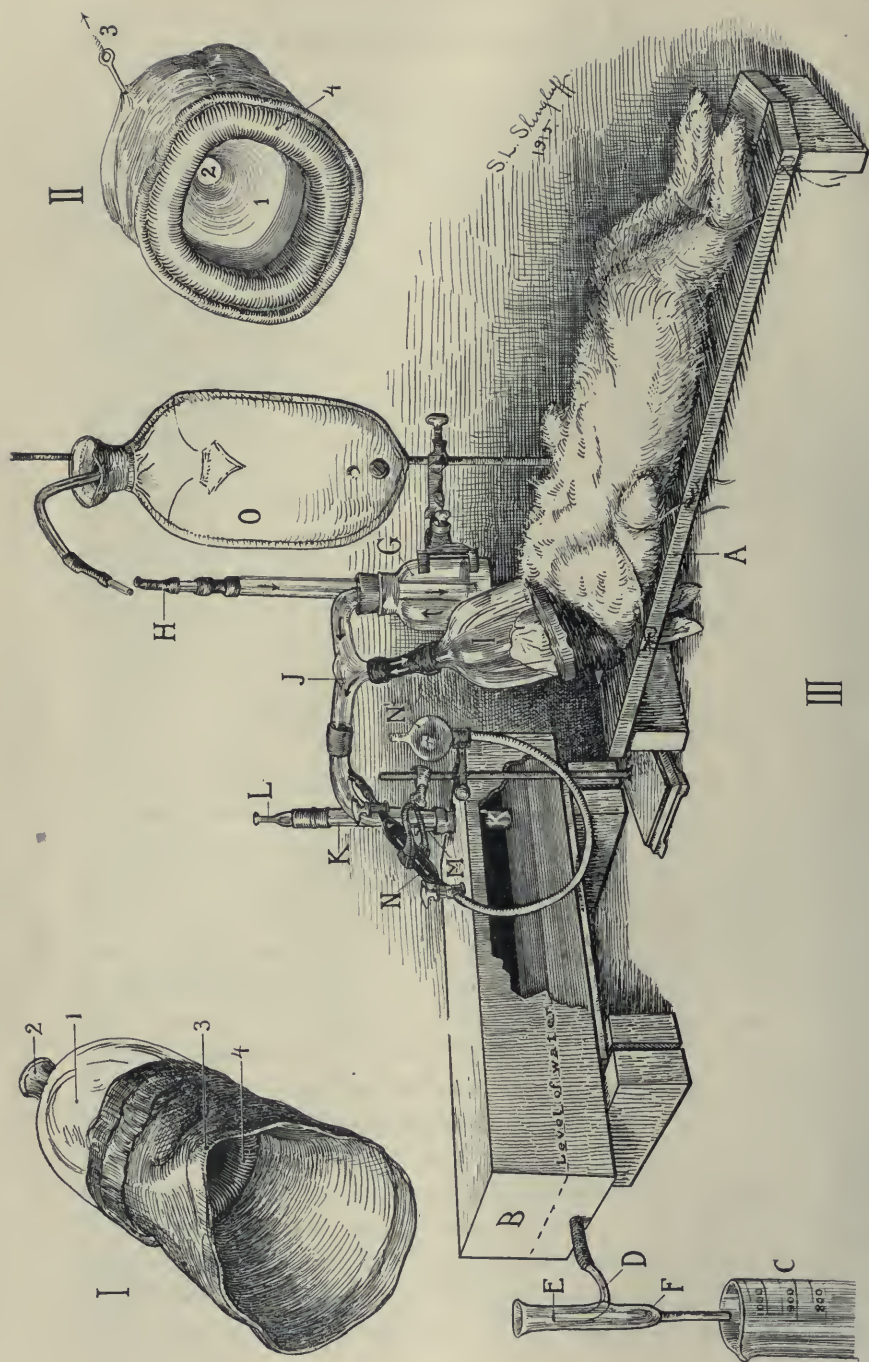


FIG. 1.

Respiratory Apparatus described in the text. I and II are other views of the helmet, showing 1, body of helmet; 2, neck of helmet; 3, rubber sleeve; 4, rubber cushion on rim of helmet.

expiration the air cannot escape through *G*, being held back by the water valve *H*. It is therefore sent through *J* to the left down the tube *K* into the tank *B*, and displaces an equivalent volume of water which overflows from *E*, through the funnel-like arrangement *F* into the cylinder *C*, and can be measured. With each respiration the process is repeated, so that the volume of the total expired air per minute can be easily measured. The rate of breathing is counted by observing the movements of the chest or the bubbling of the water valve *H*. After the animal has been breathing through the apparatus for several minutes, so that the composition of the air in *J* is uniform, a sample of the expired air is slowly withdrawn by means of the mercury gas pipette *NN'*, by turning the stop cocks and lowering the mercury reservoir *N'*. This sample is later analyzed for its oxygen and carbon dioxid contents with the well known Haldane apparatus (9). The helmet is then disconnected, and the alveolar tension of the carbon dioxid determined. This is done by measuring out a definite amount of air, usually 50 cc. in a small rubber bag, and attaching the bag to the neck of the helmet. The animal is then allowed to rebreathe the air contained in the closed system consisting of the helmet and the attached rubber bag, until the CO_2 tension is in equilibrium. This has to be determined by several trials in each experiment.

By means of the above apparatus we are thus enabled to determine, (1) the rate of respiration, (2) the volume of the respired air, (3) the alveolar CO_2 tension, and (4) the CO_2 in the respired air. We can furthermore study the (5) excitability or depression of the respiratory center by allowing the animal to breathe an atmosphere containing a given percentage of CO_2 , which is done simply by preparing such a mixture in a gasometer, filling a rubber bag *O* with it, and connecting the rubber bag with *H*. The alveolar CO_2 I have found to be on the average in the neighborhood of 6.2 per cent. The percentage of CO_2 in the expired air is variable depending as it does on the rate and volume of the respiration. My figures, however, I find agree very closely with those of Filehne and Kionka (9).

Knowing the alveolar CO_2 tension and the CO_2 percentage of the expired air, the alveolar ventilation or the efficient respiration can be easily computed. If we call the alveolar CO_2 per cent Q , the CO_2 percentage of the expired air P , the total volume of air respired V , and the efficient respiration or alveolar ventilation E , then we have

$$E \times Q = V \times P$$

$$\therefore E = \frac{V \times P}{Q}$$

Knowing the alveolar ventilation E , and the total ventilation V , the *dead space* (S), can be approximately determined by dividing the difference by the rate of respiration.

In other words

$$S \text{ (dead space)} = \frac{V - E}{R \text{ (rate of resp. per min.)}}$$

The indirect effect of the circulation on respiration will be discussed in another paper, on the action of opium alkaloids on the circulation. It may be stated in this place that in the present research the doses of the drugs used were for the most part too small to produce appreciable variations from this source.

REMARKS ON THE METHOD

I have found the above method of studying the respiration by far the most satisfactory. It dispenses with anesthesia or operations, which are fatal disturbing factors in a research such as the present one. To obtain accurate data, however, care must be taken to eliminate the nervous element due to the excitement or fright of the animal. To do this it is advisable, after tying down the animal, to keep it covered so as to maintain its temperature constant, and allow it to rest and compose itself in a quiet room with a uniform temperature. I generally wait from a half hour to an hour or even more before beginning the experiment proper. In this way the initial excitement stage subsides, and uniform normal readings can be obtained. I have found, moreover, that animals become accustomed or "trained" for the ex-

perimentation so that by using the same animals from time to time, the nervous element is still more easily overcome. The rabbit is by far the best animal to use for all respiratory experiments, but as some opium alkaloids, notably *codein*, affect these animals somewhat differently from others, I have repeated some of the experiments, using the same apparatus, on puppies. In administering a drug it is furthermore preferable to inject it subcutaneously, rather than intravenously, as the sudden introduction of an active alkaloid into the system by the latter method is liable to produce too sudden a shock to the nervous system, resulting in an excessive depression or some other undesirable effect.

The following protocol illustrates well, the entire unreliability of the successive volume and rate readings in an animal which has been frightened and rendered nervous by improper handling, without having been allowed sufficient time to compose itself:

Experiment, January 26, 1915. Rabbit, 1750 gms.

Rate of Respiration per min.	Volume of Expired Air cc.
64.....	480
60.....	360
60.....	310
60.....	325
54.....	550
60.....	450
68.....	280
60.....	220
68.....	410
68.....	330
62.....	200
70.....	350
60.....	250
60.....	310
70.....	325
70.....	400
60.....	330

The following protocol on the other hand illustrates well the remarkable constancy in readings obtained from an animal which has been tied carefully down as above described, in a quiet room, covered with a blanket to keep its temperature uniform and allowed to quiet itself for about half an hour before beginning the

experiment. It further illustrates the inappreciable effect of a hypodermic injection of Locke's Solution, showing that the pain from the needle does not affect the readings. The animal was kept in position for nearly 5 hours.

Experiment, February 11, 1915. Rabbit, 1620 gms.

12.15 p.m. Animal is tied on its back, gently covered with a woolen cloth, and allowed to rest in a quiet room.

		Rate of resp. per min.	Vol. of Exp. Air cc.
12.45 p.m.	Breathing air of room	48	450
	Breathing air with CO ₂ 5 %	48	600
1.45 p.m.	Breathing air of room	48	450
	Breathing air with CO ₂ 5%	48	550
1.50 p.m.	Injected under skin of abdomen 4 cc. of Locke Solution		
2.20 p.m.	Breathing air of room	48	450
	Breathing air with CO ₂ 5%	48	550
3.30 p.m.	Breathing air of room	48	450
	Breathing air with CO ₂ 5%	48	550
3.50 p.m.	Breathing air of room	48	450
	Breathing air with CO ₂ 5%	46	550
5.00 p.m.	Breathing air of room	50	450
	Breathing air with CO ₂ 5%	48	550

ALKALOIDS TESTED

In the present work my attention was confined to the following seven opium alkaloids: morphin, codein, narcotin, papaverin, narcein, thebain, and cryptopin. The first six of these as I have already pointed (2) out are the principal constituents of opium in point of quantity. I have also studied the behavior of some combinations of these, as well as of a mixture of the chlorides of the total opium alkaloids prepared by Sahli (10). The products used were the purest obtainable, and the same as those employed by me in the study of the coronary circulation. Some of the derivatives of morphin, such as heroin, etc., are of such importance in respiratory therapeutics that they will be dealt with from a somewhat different point of view in a separate paper.

THE ACTION OF MORPHIN

The influence of morphin on the respiration as studied by the method above described, varies with its dosage. When a full dose of morphin, is injected into a rabbit, the rate is most markedly slowed, the total volume of air respired is diminished, and the alveolar ventilation is also diminished. The volume of air expired with each respiration may sometimes be increased, and this has been favorably regarded by earlier writers. The total volume of air respired, is however, as already explained, not indicative of the *efficiency* of the respiration, and it is the *alveolar* ventilation that is the criterion in this respect. The excitability of the respiratory center to CO_2 is markedly diminished, and may be entirely paralyzed. The dead space is slightly increased pointing to a broncho-dilatation. These effects may be observed after doses of from one to five milligrams per kilogram of a rabbit, and in puppies after even smaller doses. The following protocols will serve as illustrations.

Experiment, March 22, 1915. White rabbit, 1500 gms.

11.30 a.m. Animal placed in apparatus, covered, and left in a quiet room.

		Rate per min.	Vol. per min. cc.	Vol. per resp. cc.
1.10 p.m.	Breathing air.	50	430	8.6
	Breathing 2.5% CO_2 ..	48	500	10.4
	Breathing 5% CO_2 ..	48	600	14.1
	Alveolar CO_2 6.40 per cent.			
	CO_2 of expired air 1.52 per cent.			
	Alveolar ventilation 102.1 cc.			
	Dead space 6.5 cc.			

1.20 p.m. Injected morphin sulphate 2 mgms. subcutaneously.

		Rate per min.	Volume per min. cc.	Volume per resp. cc.
2.00 p.m.	Breathing air.	34	310	9.1
	Breathing CO_2 2.5%..	34	350	10.2
	Breathing CO_2 5%...	34	420	12.3
	Alveolar CO_2 7.20 per cent.			

- 2.00 p.m. CO₂ of expired air 1.73 per cent.
 Alveolar ventilation 74.4 cc.
 Dead space 6.9 cc.
- 2.20 p.m. Injected morphin sulphate 2 mgms. subcutaneously.

	<i>Rate per min.</i>	<i>Volume per min. cc.</i>	<i>Volume per resp. cc.</i>
2.35 p.m. Breathing air.....	28	250	8.9
Breathing CO ₂ 2.5%..	28	320	11.4
Breathing CO ₂ 5%...	28	400	14.2
Alveolar CO ₂ 7.50 per cent.			
CO ₂ of expired air 1.61 per cent.			
Alveolar ventilation 53.6 cc.			
Dead space 7.0 cc.			

- 3.00 p.m. Injected morphin sulphate, 2 mgms. subcutaneously.

	<i>Rate per min.</i>	<i>Volume per min. cc.</i>	<i>Volume per resp. cc.</i>
3.15 p.m. Breathing air.....	26	200	7.6
Breathing CO ₂ 2.5%..	26	200	7.6
Breathing CO ₂ 5%...	28	250	8.9
Alveolar CO ₂ 7.50 per cent.			
CO ₂ of expired air 1.10 per cent.			
Alveolar ventilation 29.3 cc.			
Dead space 6.5 cc.			

- 3.35 p.m. Injected morphin sulphate, 2 mgms. subcutaneously.

	<i>Rate per min.</i>	<i>Volume per min. cc.</i>	<i>Volume per resp. cc.</i>
4.00 p.m. Breathing air.....	26	200	7.6
Breathing CO ₂ 2.5%..	26	200	7.6
Breathing CO ₂ 5 %..	26	200	7.6
Alveolar CO ₂ 7.6 per cent.			
Respiratory CO ₂ 0.8 per cent.			
Alveolar ventilation 21 cc.			
Dead space 6.8 cc.			

Experiment, April 14, 1915. Black puppy, 1600 gms.

- 12.30 p.m. Animal placed on apparatus, covered, and left in quiet room.

		Rate per min.	Volume per min. cc.	Volume per resp. cc.
2.00 p.m.	Breathing air.....	28	400	14.2
	Breathing CO ₂ 2.5% .	28	600	21.4
	Alveolar CO ₂ 6.1 per cent.			
	CO ₂ of expired air 2.2 per cent.			
	Alveolar ventilation 131.1 cc.			
	Dead space 9.5 cc.			
2.10 p.m.	Injected morphin sulphate, 0.5 mgms. subcutaneously.			
		Rate per min.	Volume per min. cc.	Volume per resp. cc.
2.30 p.m.	Breathing air.....	22	350	15.9
	Breathing CO ₂ 5.%...	22	350	15.9
	Alveolar CO ₂ 6.30 per cent.			
	CO ₂ of expired air 1.70 per cent.			
	Alveolar ventilation 94.4 cc.			
	Dead space 11.5 cc.			

The above doses of morphin are entirely too large. If smaller doses of morphin are administered, quite a different picture results. After doses of about 0.1 mgm. per kilo weight of rabbit or puppy, the rate of respiration is still markedly slowed, but the volume of the air respired is increased, and, what is more important, the alveolar ventilation is also increased,—a very much desired result from a therapeutic point of view. The respiratory center is still depressed but not to such a high degree. These effects may be illustrated by the following protocols.

Experiment, April 29, 1915. Rabbit, 1200 gms.

10.00 a.m. Animal placed on apparatus, covered, and in a quiet room.

		Rate per min.	Volume per min. cc.	Volume per resp. cc.
10.30 a.m.	Breathing air.....	60	360	6.0
	Breathing CO ₂ 5%...	60	500	8.3
	Alveolar CO ₂ 6.30 per cent.			
	CO ₂ of expired air 1.62 per cent.			
	Alveolar ventilation 92.4 cc.			
	Dead space 4.4 cc.			
10.40 a.m.	Injected morphin sulphate, 0.14 mgms. subcutaneously.			

		<i>Rate per min.</i>	<i>Volume per min. cc.</i>	<i>Volume per resp. cc.</i>
11.10 a.m.	Breathing air.....	48	400	8.3
	Breathing CO ₂ 5%...	48	500	10.4
	Alveolar CO ₂ 6.30 per cent.			
	CO ₂ of expired air 1.74 per cent.			
	Alveolar ventilation 110.4 cc.			
	Dead space 6.0 cc.			
11.25 a.m.	Injected morphin sulphate, 0.13 mgms. subcutaneously.			
		<i>Rate per min.</i>	<i>Volume per min. cc.</i>	<i>Volume per resp. cc.</i>
12.45 p.m.	Breathing air.....	44	380	8.6
	Breathing CO ₂ 5%...	44	450	10.2
	Alveolar CO ₂ 6.80 per cent.			
	CO ₂ of expired air 1.74 per cent.			
	Alveolar ventilation 97.2 cc.			
	Dead space 6.4 cc.			
1.05 p.m.	Injected morphin sulphate, 0.13 mgms. subcutaneously.			
		<i>Rate per min.</i>	<i>Volume per min. cc.</i>	<i>Volume per resp. cc.</i>
2.05 p.m.	Breathing air.....	46	400	8.6
	Breathing CO ₂ 5%...	46	450	9.7
	Alveolar CO ₂ 6.00 per cent.			
	CO ₂ of expired air 1.72 per cent.			
	Alveolar ventilation 114.3 cc.			
	Dead space 6.2 cc.			

Experiment, May 3, 1915. Puppy, 1900 gms.

1.00 p.m. Animal tied down, covered, and left in quiet.

		<i>Rate per min.</i>	<i>Volume per min. cc.</i>	<i>Volume per resp. cc.</i>
1.45 p.m.	Breathing air.....	22	400	18.1
	Breathing CO ₂ 2.5%..	20	500	25
	Alveolar CO ₂ 6.0 per cent.			
	CO ₂ of expired air 2.16 per cent.			
	Alveolar ventilation 144 cc.			
	Dead space 11.6 cc.			
2.00 p.m.	Injected morphin sulphate, 0.2 mgms. subcutaneously.			

		Rate per min.	Volume per min. cc.	Volume per resp. cc.
2.45 p.m.	Breathing air	16	425	26.5
	Breathing CO ₂ 2.5% . .	18	480	26.6
	Alveolar CO ₂ 5.9 per cent.			
	CO ₂ of expired air 2.19 per cent.			
	Alveolar ventilation 159.3 cc.			
	Dead space 16.6 cc.			
3.00 p.m.	Injected morphin sulphate, 0.2 mgms. subcutaneously.			
		Rate per min.	Volume per min. cc.	Volume per resp. cc.
3.25 p.m.	Breathing air	16	410	25.6
	Breathing CO ₂ 2.5% . .	18	475	26.4
	Alveolar CO ₂ 6.0 per cent.			
	CO ₂ of expired air 2.39 per cent.			
	Alveolar ventilation 163.3 cc.			
	Dead space 15.4 cc.			
3.30 p.m.	Injected morphin sulphate, 0.2 mgms. subcutaneously.			
		Rate per min.	Volume per min. cc.	Volume per resp. cc.
4.00 p.m.	Breathing air	16	410	25.6
	Breathing CO ₂ 2.5% . .	18	475	26.4
	Alveolar CO ₂ 6.4 per cent.			
	CO ₂ of expired air 2.21 per cent.			
	Alveolar ventilation 141.8 cc.			
	Dead space 17.3 cc.			

In this connection it may be well to give one or two brief protocols showing how greatly the effect of morphin may be distorted in tracheatomized or anesthetized animals.

Experiment, February 26, 1915. Rabbit, 1450 gm. Animal is first put under ether, a tracheal tube is inserted, and the anesthetic is removed.

11.15 a.m. Rate 86 per min. Vol. 520 cc. per min.
 11.30 a.m. Injected morphin sulphate 4 mgms. subcutaneously.
 12.15 p.m. Rate 80 per min. Vol. 410 cc. per min.
 12.45 p.m. Injected morphin sulphate 4 mgms. subcutaneously.

1.15 p.m. Rate 80 per min. Vol. 370 cc. per min.

2.30 p.m. Rate 80 per min. Vol. 370 cc. per min.

It will be noted in this experiment that the irritation of the tracheal tube and the wound tend to keep the rate of respiration greater than it would otherwise be.

Experiment, January 26, 1915. Rabbit, 1200 gms.

12.00 m. Rabbit under chloral-urethane anesthesia, with tracheal tube inserted. Rate 45 per min.; vol. 120 cc. per min.; breathing air. Rate 46 per min.; vol. 165 cc. per min.; breathing CO₂ 10 per cent.

12.30 p.m. Injected subcutaneously morphin sulphate 4 mgms.

1.00 p.m. Rate 36 per min.; vol. 120 cc. per min.; breathing air. Rate 36 per min.; vol. 150 cc. per min.; breathing CO₂ 10 per cent.

1.45 p.m. Spasmodic movements.

1.50 p.m. Rate 34 per min.; vol. 95 cc. per min.; breathing air. Rate 34 per min.; vol. 140 cc. per min.; breathing CO₂ 10 per cent.

Here we note the marked shallowness of the animal's breathing even before the injection of the drug, and the poor response of the center even to a strong atmosphere of CO₂ (10 per cent).

THE ACTION OF CODEIN

The effect of codein on the respiration is in some respects similar to that of morphin, in other respects it is very different. In the first place the dosage is, as is well known, much greater. In rabbits doses of about 1 to 2 and sometimes more milligrams per kilogram weight will generally cause a slight slowing of the rate of breathing, the total volume output is generally not much diminished, and may even be increased; the alveolar ventilation is certainly not diminished, and the sensitiveness of the respiratory center to CO₂ is very slightly decreased. Larger doses instead of further depressing the center, increase its sensitiveness, so that there is a distinctly more efficient alveolar ventilation. The animal is in a weakly narcotic state, from which it can be easily put into a state of hyperexcitability by further administration of the drug. Very large doses of codein, 20 mgms. or more

per kilo will produce convulsions in rabbits. It is a well known fact noted by the earlier workers with codein, that that drug is more toxic to rabbits than other animals. In dogs the depressive action of codein on the respiration is more marked, but as a whole the effect of codein is the same as in rabbits. The following protocols will illustrate some of the points just mentioned.

Experiment, April 1, 1915. Rabbit, 1620 gms.

10.30 a.m. Animal fixed on apparatus and allowed to rest.

		Rate per min.	Volume per min. cc.	Volume per resp. cc.
11.00 a.m.	Breathing air.....	44	300	6.8
	Breathing CO ₂ 2.5%..	44	400	9.0
	Alveolar CO ₂ 6.6 per cent.			
	CO ₂ of expired air 1.00 per cent.			
	Alveolar ventilation 45.5 cc.			
	Dead space 5.8 cc.			

11.10 a.m. Injected codein phosphate 2 mgms. subcutaneously.

		Rate per min.	Volume per min. cc.	Volume per resp. cc.
11.30 a.m.	Breathing air.....	40	300	7.5
	Breathing CO ₂ 2.5%..	40	420	10.5
	Alveolar CO ₂ 6.8 per cent.			
	CO ₂ of expired air 1.32 per cent.			
	Alveolar ventilation 58.2 cc.			
	Dead space 6.0 cc.			

11.37 a.m. Injected codein phosphate, 4 mgm. subcutaneously.

		Rate per min.	Volume per min. cc.	Volume per resp. cc.
12.00 m.	Breathing air.....	42	375	8.9
	Breathing CO ₂ 2.5%...	40	375	9.4
	Breathing CO ₂ 5%.....	48	550	11.4
	Alveolar CO ₂ 7.0 per cent.			
	CO ₂ expired air 1.0 per cent.			
	Alveolar ventilation 53.6 cc.			
	Dead space 7.6 cc.			

Animal is narcotized, but is easily awakened and excited.

12.10 p.m. Injected codein phosphate 6 mg. subcutaneously.

		Rate per min.	Volume per min. cc.	Volume per resp. cc.
12.30 p.m.	Breathing air.....	34	260	7.6
	Breathing CO ₂ 2.5%.....	34	400	11.7
	Alveolar ventilation	45.4 cc.		
	Dead space	6.1 cc.		

		Rate per min.	Volume per min. cc.	Volume per resp. cc.
1.00 p.m.	Breathing air.....	34	300	8.8
	Breathing CO ₂ 2.5%.....	34	420	12.5
	Alveolar CO ₂	7.4 per cent.		
	CO ₂ of expired air	1.6 per cent.		
	Alveolar ventilation	64.8 cc.		
	Dead space	6.9 cc.		

Experiment, April 19, 1915. Puppy, 1650 gms.

11.30 a.m. Animal placed on board and allowed to rest.

		Rate per min.	Volume per min. cc.	Volume per resp. cc.
12.30 p.m.	Breathing air.....	24	350	15
	Breathing CO ₂ 2.5%....	24	560	23.3
	Alveolar CO ₂	6.3 per cent.		
	CO ₂ of expired air	1.93 per cent.		
	Alveolar ventilation	107.5 cc.		
	Dead space	10.1 cc.		

1.35 p.m. Inject codein phosphate 1.5 mg. subcutaneously.

		Rate per min.	Volume per min. cc.	Volume per resp. cc.
2.35 p.m.	Breathing air.....	18	350	19.4
	Breathing CO ₂ 2.5%....	18	480	26.6
	Alveolar ventilation	106.6 cc.		
	Dead space	13.5 cc.		

3.00 p.m. Injected codein phosphate 1.5 mgms. subcutaneously.

		Rate per min.	Volume per min. cc.	Volume per resp. cc.
3.30 p.m.	Breathing air.....	18	300	16.6
	Breathing CO ₂ 2.5%....	18	350	19.4
	Breathing CO ₂ 5 %....	20	400	20.0

3.30 p.m. Alveolar CO_2 6.7 per cent.
 — CO_2 of expired air 1.96 per cent.
 Alveolar ventilation 87.7 cc.
 Dead space 11.7 cc.
 Dog taken off very slightly narcotized.

THE ACTION OF NARCOTIN

This alkaloid is in point of quantity next to morphin the chief alkaloid of opium (11). As its narcotic and analgesic properties are of very little value, it received but very little attention from pharmacologists and therapeutists until the work of Straub (12) on the synergism of morphin and narcotin. Straub regards this alkaloid as "unwirksam" or inert, but he finds that the action of morphin on respiration and other functions is very much intensified by combining it with narcotin, which phenomenon he speaks of as "potentiation." This work was severely criticized by Meissner (13), who claimed that narcotin is not at all inactive, and that the effect of a mixture of the two alkaloids, is an arithmetical sum of their individual properties. The question of the combined action of morphin and narcotin I shall treat of later in the paper. As regards the effect of narcotin itself on the respiration, our experiments certainly show that narcotin is not at all inert, but acts as a stimulant to the respiratory center. The rate of breathing may be slightly decreased, the respiratory center, however, is distinctly excited instead of being depressed or paralyzed. The following protocol will serve as an illustration of the action of narcotin:

Experiment 16, 1915. Rabbit, 920 gms.

10.30 a.m. Rabbit placed in position, and allowed to quiet itself.

		Rate per min.	Volume per min. cc.	Volume per resp. cc.
11.00 a.m.	Breathing air.....	50	300	6.0
	Breathing CO_2 5%.....	50	400	8.0
	Alveolar CO_2 6.4 per cent.			
	CO_2 of expired air 1.70 per cent.			

11.00 a.m.	Alveolar ventilation 79.7 cc. Dead space 4.4 cc.		
11.45 a.m.	Injected narcotin hydrochloride, 2 gms. subcutaneously.		
	<i>Rate per min.</i>	<i>Volume per min. cc.</i>	<i>Volume per resp. cc.</i>
12.15 p.m.	Breathing air..... 42	300	7.1
	Breathing CO ₂ 5%..... 44	440	10.0
	Alveolar CO ₂ 7.3 per cent. CO ₂ of expired air 1.98 per cent. Alveolar ventilation 81.3 cc. Dead space 5.3 cc.		
12.45 p.m.	Injected narcotin hydrochloride, 2 mgms. subcutaneously.		
	<i>Rate per min.</i>	<i>Volume per min. cc.</i>	<i>Volume per resp. cc.</i>
2.15 p.m.	Breathing air..... 42	290	6.9
	Breathing CO ₂ 5%..... 44	400	9.0
	Alveolar CO ₂ 6.6 per cent. CO ₂ of expired air 1.90 per cent. Alveolar ventilation 81.8 cc. Dead space 5 cc.		
2.40 p.m.	Injected narcotin hydrochloride, 4 mgms. subcutaneously.		
	<i>Rate per min.</i>	<i>Volume per min. cc.</i>	<i>Volume per resp. cc.</i>
3.00 p.m.	Breathing air..... 42	420	10
	Breathing CO ₂ 5%..... 44	550	12.4
	Alveolar CO ₂ 7.3 per cent. CO ₂ of expired air 2.1 per cent. Alveolar ventilation 122.5 cc. Dead space 7.0 cc. Animal taken off table in good condition, not narcotized.		

THE ACTION OF PAPAVERIN

The behavior of papaverin is interesting in view of its distinct narcotic and analgesic properties noted as far back as 1869 by Baxt (14), and its action on animals and in man observed by Pal (15). I have in a preceding communication called attention

to its pronounced influence on the coronary arteries and the coronary circulation.

On respiration it is interesting to find, that papaverin while slightly slowing the rate, exerts a distinctly stimulating effect. This is well illustrated by the following protocol:

Experiment, March 6, 1915. Rabbit, 1250 gms.

10.30 a.m. Animal placed on board, and allowed to quiet itself.

	<i>Rate per min.</i>	<i>Volume per min. cc.</i>	<i>Volume per resp. cc.</i>
11.00 a.m. Breathing air	60	430	7.1
Breathing CO ₂ 5%	58	550	9.4
Alveolar CO ₂ 6.6 per cent.			
CO ₂ of expired air 1.5 per cent.			
Alveolar ventilation 99.7 cc.			
Dead space 5.5 cc.			

11.40 a.m. Injected papaverin hydrochloride, 2 mgms. subcutaneously.

	<i>Rate per min.</i>	<i>Volume per min. cc.</i>	<i>Volume per resp. cc.</i>
12.15 p.m. Breathing air	50	450	9.0
Breathing CO ₂ 5%	48	550	11.4
Alveolar CO ₂ 7.1 per cent.			
CO ₂ of expired air 1.7 per cent.			
Alveolar ventilation 107.7 cc.			
Dead space 6.8 cc.			

1.15 p.m. Injected papaverin hydrochloride, 2 mgms. subcutaneously.

	<i>Rate per min.</i>	<i>Volume per min. cc.</i>	<i>Volume per resp. cc.</i>
Breathing air	50	440	8.8
Breathing CO ₂ 5%	48	560	11.6
Alveolar CO ₂ 6.8 per cent.			
CO ₂ of expired air 2.9 per cent.			
Alveolar ventilation 187.6 cc.			
Dead space 5.0 cc.			

3.15 p.m. Animal shows signs of definite narcotization.

	Rate per min.	Volume per min. cc.	Volume per resp. cc.
4.45 p.m. Breathing air.....	60	550	9.1
Breathing CO ₂ 5%.....	56	600	10.7
Alveolar CO ₂ 7.7 per cent.			
CO ₂ of expired air 2.35 per cent.			
Alveolar ventilation 167.8 cc.			
Dead space 6.3 cc.			
Animal still a little narcotized.			

THE ACTION OF NARCEIN, THEBAIN AND CRYPTOPIN

The action of these alkaloids can be dealt with together. These constituents of opium are entirely devoid of narcotic, sedative or analgesic properties. Thebain, as is well known, is in its pharmacological action a convulsant drug almost indistinguishable from strychnin. On the respiratory center these drugs either have no effect, or they act as excitants as may be exemplified by the following experiment with cryptopin.

Experiment, March 24, 1915. Rabbit, 1380 gms.

1.00 p.m. Animal placed in position, and allowed to get quiet.

	Rate per min.	Volume per min. cc.	Volume per resp. cc.
3.30 p.m. Breathing air.....	64	500	7.8
Breathing CO ₂ 2.5%....	64	600	9.0
Alveolar CO ₂ 8.6 per cent.			
CO ₂ of expired air 2.4 per cent.			
Alveolar ventilation 139.9 cc.			
Dead space 5.6 cc.			

3.40 p.m. Injected cryptopin hydrochloride, 2 mgms.

	Rate per min.	Volume per min. cc.	Volume per resp. cc.
4.00 p.m. Breathing air.....	68	500	7.5
Breathing CO ₂ 2.5%..	68	600	8.8
Alveolar CO ₂ 8.6 per cent.			
CO ₂ of expired air 2.65 per cent.			
Alveolar ventilation 154.0 cc.			
Dead space 5.0 cc.			

4.10 p.m. Injected cryptopin hydrochloride, 4 mgms.

	<i>Rate per min.</i>	<i>Volume per min. cc.</i>	<i>Volume per resp. cc.</i>
4.45 p.m. Breathing air	68	500	7.5
Breathing CO ₂ 2.5%	68	600	8.8
Alveolar CO ₂ 7.9 per cent.			
CO ₂ of expired air 2.6 per cent.			
Alveolar ventilation 164.5 cc.			
Dead space 5.0 cc.			

Narcein, regarded by v. Schröder as an inert substance, was found by me when 20 mgms. of it were injected subcutaneously into a guinea pig weighing 200 gms. to produce rapid and irregular respiration, followed quickly by convulsions and opisthotonos which continued for a couple of hours, and ended in death.

The following table shows the hyperexcitability of the respiratory center produced by that drug.

Experiment, March 17, 1915. Rabbit, 2000 gms.

3.00 p.m.	Rate of resp. 38 per min.; vol. 400 cc. per min.; breathing air. Rate of resp. 38 per min.; vol. 500 cc. per min.; breathing CO ₂ 5%.
3.10 p.m.	Injected narcein, 2 mgms. subcutaneously.
3.30 p.m.	Rate of resp., 36 per min.; vol. 400 cc. per min.; breathing air. Rate of resp., 36 per min.; vol. 600 cc. per min.; breathing CO ₂ 5 per cent.
3.40 p.m.	Injected narcein, 2 mgms.
4.10 p.m.	Rate of resp., 36 per min.; vol. 400 cc. per min.; breathing air. Rate of resp., 36 per min.; vol. 700 cc. per min.; breathing CO ₂ 5 per cent.
4.30 p.m.	Injected narcein, 4 mgms.
4.40 p.m.	Rate of resp., 36 per min.; vol. 400 cc. per min.; breathing air. Rate of resp., 36 per min.; vol. 700 cc. per min.; breathing CO ₂ 5 per cent.
4.45 p.m.	Injected narcein, 10 mgms.
5.10 p.m.	Rate of resp. 38 per min.; vol. 400 cc. per min.; breathing air. Rate of resp., 38 per min.; vol. 700 cc. per min.; breathing CO ₂ 5 per cent.
5.15 p.m.	Animal very hyperexcitable, and exhibits slight convulsions.

THE ACTION OF COMBINATIONS OF OPIUM ALKALOIDS

Various combinations of opium derivatives, and more especially combinations of morphin with other opium alkaloids have in the last few years been claimed to exhibit more efficacious and fewer ontoward properties than morphin alone, and accordingly a number of such preparations with fanciful names have been put by drug firms on the market. It was therefore interesting, and from a practical point of view, important to investigate the action of some of these on the respiratory function. The most important pharmacological work on such opium combinations has been that of Straub, Faust, and Sahli.

Straub (12) recommends a combination of equimolecular weights of morphin and narcotin ("Narcophin") as a much more efficient and less depressant opiate than morphin alone for respiratory conditions.

Faust (16) considers a mixture of opium alkaloids in the following proportions as most efficient for the purpose ("Laudanon"):

	<i>mgm.</i>		<i>mgm.</i>		<i>mgm.</i>
Morphin.....	10	Codein.....	1	Thebain.....	0.5
Narcotin.....	6	Papaverin.....	2	Narcein.....	0.5

Sahli (10) experimented with a soluble preparation consisting of the chlorides of the total opium alkaloids, minus the gums, sugars, protein matter, and other by-products of the crude drug ("Pantopon").

ACTION OF MORPHIN PLUS NARCOTIN

The following two protocols, of experiments performed on the same animal on two different days will illustrate well the difference between the action of morphin alone, and morphin in combination with narcotin.

Experiment A, March 15, 1915. Rabbit, 920 gms.

11.00 a.m. Animal placed on apparatus.

12.00 m. Rate 76; vol. 300 cc.; breathing air. Rate, 72; vol. 380 cc.; breathing CO₂ 2.5 per cent. Rate 76; vol. 600 cc.; breathing CO₂ 10 per cent.

- 12.00 m. Alveolar CO_2 6.7 per cent.
CO₂ of expired air 2.0 per cent.
Alveolar ventilation 89.5 cc.
Dead space 2.8 cc.
- 12.45 p.m. Injected morphin sulphate, 1 mgms. subcutaneously.
- 1.15 p.m. Rate 42; vol. 200 cc.; breathing air. Rate 42; vol. 210 cc.; breathing CO₂ 2.5 per cent. Rate 42; vol. 260 cc.; breathing CO₂ 10 per cent.
Alveolar CO₂ 6.1 per cent.
CO₂ of expired air 1.4 per cent.
Alveolar ventilation 45.8 cc.
Dead space 3.4 cc.
- 1.30 p.m. Injected morphin sulphate, 1 mgms. subcutaneously.
- 2.15 p.m. Rate 32; vol. 160 cc.; breathing air. Rate 32; vol. 160 cc.; breathing CO₂ 2.5 per cent. Rate 32; vol. 160 cc.; breathing CO₂ 10 per cent.
Alveolar CO₂ 6.1 per cent.
CO₂ of expired air 1.8 per cent.
Efficient respiration 47.2 cc.
Dead space 3.5 cc.
Rabbit distinctly narcotized.

Experiment B, March 17, Rabbit, 920 gms.

- 11.45 a.m. Animal placed in apparatus.
- 12.30 p.m. Rate 60; vol. 400 cc.; breathing air. Rate 60; vol. 500 cc.; breathing CO₂ 5 per cent.
Alveolar CO₂ 6.5 per cent.
CO₂ of expired air 1.6 per cent.
Alveolar ventilation 98.4 cc.
Dead space 5.0 cc.
- 12.40 p.m. Injected morphin-narcotin meconate, 2 mgms. subcutaneously.
- 2.00 p.m. Rate 48; vol. 350; breathing air. Rate 46; vol. 440; breathing CO₂ 5 per cent.
Alveolar CO₂ 6.6 per cent.
CO₂ of expired air 1.6 per cent.
Alveolar ventilation 86.2 cc.
Dead space 5.4 cc.
- 2.30 p.m. Injected morphin-narcotin meconate, 2 mgms. subcutaneously.

- 3.00 p.m. Rate 44; vol. 370 cc.; breathing air. Rate 44; vol. 460 cc.; breathing CO₂ 5 per cent.
Alveolar CO₂ 7.4 per cent, 7.6 per cent.
CO₂ of expired air 1.7 per cent.
Alveolar ventilation 83.8 cc.
Dead space 6.5 cc.
- 3.30 p.m. Injected morphin-narcotin meconate 2 mgms. subcutaneously.
- 3.50 p.m. Rate 38; vol. 375 cc.; breathing air. Rate 38; vol. 410 cc.; breathing CO₂ 5 per cent.
Alveolar CO₂ 7.5 per cent, 7.2 per cent.
CO₂ of expired air 1.6 per cent.
Efficient ventilation 82.2 cc.
Dead space, 7.7 cc.
- 4.15 p.m. Injected morphin-narcotin meconate, 2 mgms. subcutaneously.
- 4.45 p.m. Rate 38; vol. 340 cc.; breathing air. Rate 40; vol. 400 cc.; breathing CO₂ 5 per cent.
Alveolar 6.8 per cent.
CO₂ of expired air 1.7 per cent.
Efficient ventilation 85.0 cc.
Dead space 6.7 cc.
Animal distinctly narcotized.

On comparing experiments A and B it is evident that morphin alone is much more depressant to the respiratory center than morphin given together with narcotin, as is shown by the poorer ventilation and weaker response to breathing air containing 10 per cent of CO₂. Whereas in Experiment A, the animal's center was completely paralyzed after the injection of 2 mgms. of morphin, we find in experiment B the same animal, after having gone through a morphin injection 2 days previously, still responding to inhalation of 5 per cent CO₂, following a total administration of 8 mgms. of morphin-narcotin meconate which contains an equivalent of nearly 4 mgms. of morphin. So far these results corroborate Straub's observations.

I have obtained similar results with other combinations of morphin and narcotin, for instance in proportions of 1:1 and 10:6, in both rabbits and puppies. So that there is no doubt as

to the difference in action between morphin alone and morphin plus narcotin. The explanation of the phenomenon is however not due to any mysterious "potentiation" of the properties of morphin but is simply due to a *summation* of the morphin and narcotin effects, as can be readily seen by recalling the stimulant action of narcotin described above, and in this respect our experiments seem to confirm Meissner's view.

THE ACTION OF OTHER COMBINATIONS

The action of other combinations of opium alkaloids on the respiration can also be completely explained by bearing in mind the properties of the individual alkaloids. Thus the following protocol shows the far less depressant and more efficient effect of Faust's mixture, as compared with that of morphin alone.

Experiment, April 12, 1915. Rabbit, 1100 gms.

- 12.00 m. Animal tied down and allowed to rest.
- 12.45 p.m. Rate 68; vol. 400; breathing air. Rate 68; vol. 500;
breathing CO₂ 2.5 per cent.
Alveolar CO₂ 7.4 per cent.
CO₂ of expired air 1.8 per cent.
Alveolar ventilation 98 cc.
Dead space 4 cc.
- 12.55 p.m. Injected Faust's combination 4 mgms. (containing morphin 2 mgms.)
- 1.15 p.m. Rate 56; vol. 400 cc.; breathing air. Rate 54; vol. 500 cc.; breathing CO₂ 2.5 per cent.
Alveolar CO₂ 6.3 per cent.
CO₂ of expired air 1.51 per cent.
Alveolar ventilation 95 cc.
Dead space 5 cc.
- 1.25 p.m. Injected 4 mgms. (= morphin 2 mgms.).
- 1.45 p.m. Rate 44; vol. 300; breathing air. Rate 44; vol. 400; breathing CO₂ 2.5 per cent.
Alveolar CO₂ 7.4 per cent.
CO₂ of expired air 1.79 per cent.
Alveolar ventilation 73 cc.
Dead space 5 cc.

2.15 p.m. Injected 4 mgms. (= morphin 2 mgms.).

2.40 p.m. Rate 38; vol. 300; breathing air. Rate 38; vol. 400;
breathing CO₂ 5 per cent.

Alveolar CO₂ 7.7 per cent.

CO₂ of expired air 1.75 per cent.

Alveolar ventilation 68 cc.

Dead space 6 cc.

Again by administering morphin in combination with papaverin in the proportion of 1:1 I have been able to produce less depression in a rabbit of 1200 gms. with 10 mgms. of the mixture, than with 3 mgms. of morphin sulphate alone in a rabbit weighing 1700 gms.

ACTION OF TOTAL OPIUM ALKALOIDS

It has long been an empirical observation among clinicians that opium and its galenical preparations are less depressant to the respiration than the corresponding quantity of the morphin alkaloid given alone. It was partly with this end in view that Sahli employed a mixture of the salts of the total alkaloids, for the purpose of more exact dosage and more convenient administration. It was therefore especially desirable to compare the action of Sahli's mixture with that of morphin. The results were extremely interesting. It was found that several times as much morphin could be administered in the form of Sahli's mixture, than of morphin alone and that with more efficient and less depressant effect on the respiration. The explanation here is again, the antagonistic or corrective action of the other alkaloids present in the mixture. The two following protocols illustrate this difference in a striking fashion.

Experiment A, March 29. Rabbit, 900 gms.

12.10 p.m. Animal tied down, covered and left to quiet itself.

12.30 p.m. Rate 68; vol. 300 cc.; breathing air. Rate 64; vol. 400 cc.; breathing CO₂ 5 per cent.

Alveolar CO₂ 6.1 per cent, 6.2 per cent.

CO₂ of expired air 1.45 per cent.

Alveolar ventilation 70.7 cc.

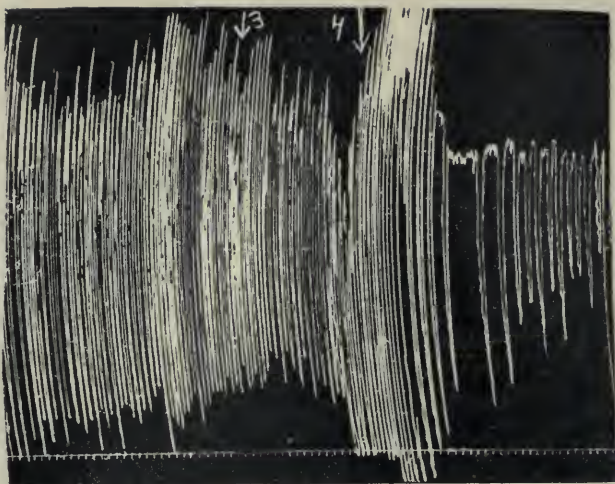
Dead space 3.4 cc.

- 12.40 p.m. Injected total opium alkaloids 2 mgms. (= 1 mgms. anhydrous morphin).
- 1.00 p.m. Rate 56; vol. 400 cc.; breathing air. Rate 54; vol. 500 cc.; breathing CO₂ 5 per cent.
Alveolar 6.4 per cent.
CO₂ of expired air 1.5 per cent.
Alveolar ventilation 98.1 cc.
Dead space 5.3 cc.
- 1.20 p.m. Injected total alkaloids 4 mgms. (= 2 mgms. anhydrous morphin)
- 1.40 p.m. Rate 42; vol. 360; breathing air. Rate 40; vol. 400, breathing CO₂ 5 per cent.
Alveolar CO₂ 7.0 per cent.
CO₂ of expired air 1.74 per cent.
Alveolar ventilation 89.5 cc.
Dead space 6.4 cc.
- 1.50 p.m. Injected total alkaloids 8 mgms. (= 4 mgms. anhydrous morphin).
- 2.10 p.m. Rate 30; vol. 200 cc.; breathing air; rate 30; vol. 290 cc.; breathing CO₂ 5 per cent.
Alveolar CO₂ 7.2 per cent.
CO₂ of expired air 2.24 per cent.
Alveolar ventilation 62.2 cc.
Dead space 4.6 cc.

Experiment B. May 29. Rabbit, 1000 gms.

- 2.15 p.m. Experiment begins.
- 2.30 p.m. Rate 56; vol. 300 cc.; breathing air. Rate 54; vol. 400 cc.; breathing CO₂ 2.5 per cent. Rate 54; vol. 520 cc.; breathing CO₂ 5 per cent.
Alveolar CO₂ 6.0 per cent.
CO₂ of expired air 1.62 per cent.
Alveolar ventilation 81 cc.
Dead space 3.9 cc.
- 2.58 p.m. Injected morphin sulphate 2 mg.
- 3.12 p.m. Rate 40; vol. 260 cc.; breathing air. Rate 40; vol. 260 cc.; breathing CO₂ 2.5 per cent. Rate 40; vol. 260 cc.; breathing CO₂ 5 per cent. Rate 40; vol. 260 cc.; breathing CO₂ 10 per cent.
Alveolar CO₂ 7.5 per cent.

B



A



FIG. 2. EXPERIMENT APRIL 26, 1915. PERFUSION OF RESPIRATORY CENTER OF A DOG BY HOOKER'S METHOD.
WT. OF DOG 7.5 KILO.

In A 1 mg. of morphin sulphate plus narcotin hydrochloride in proportions of 1:1 was introduced between arrows 1 and 2.

Note stimulation.

In B. 0.5 mg. morphin sulphate was introduced between arrows 3 and 4. Note depression.

3.12 p.m. CO₂ of expired air 2.18 per cent.

Alveolar ventilation 75.4 cc.

Dead space 4.6 cc.

Here we see 2 mgms. of morphin *completely paralyze* the respiratory center of a rabbit weighing 1000 gms. on the one hand, and on the other the respiratory center of a smaller rabbit weighing 900 gms. still responsive to CO₂ inhalation after 14 mgms. (= 7 mgms. anhydrous morphin) of the total alkaloids of opium.

ANALYSIS OF THE CENTRAL ACTION

In all of our experiments the action of opium alkaloids on the respiratory center was studied, in the first place noting the CO₂ metabolism, and secondly by observing its response to inhalations of air containing known percentages of CO₂ gas.

To make the investigation more complete, I was enabled, thanks to the kindness of Dr. D. C. Hooker of the Physiological Department of this University to study the effect of some of my drugs on the respiratory center directly. Dr. Hooker has devised and had constructed, an apparatus for maintaining the circulation of an animal's head apart from that of the rest of the body. The apparatus is virtually a pump taking the place of the heart and arranged to produce a pulse wave by means of which defibrinated or hirudinized blood is circulated through the animal's head. In this way the activity of the respiratory center can be studied by its rhythmic discharge through the phrenic nerve, thus producing movements of the diaphragm, which can be recorded on a kymograph (11). I have had the opportunity of testing in this way the action of morphin, and narcotin, individually and in combination with each other, and the observations agree completely with those obtained by the indirect method. Morphin was found to depress the center, while narcotin powerfully excites it. A combination of the two in the proportion of 1:1 gave a stimulation, showing the preponderance of the narcotin effect. Figures 2 and 3 illustrate these observations. With the same apparatus it was found that a mixture of total opium alkaloids is less depressant than morphin alone.



FIG. 3

Experiment April 27, 1915. Perfusion of respiratory center by Hooker's Method. Dog weighing 7.5 kilo. Between X and Y 1 mg. of Sahli's mixture of total opium alkaloids (≈ 0.5 mg. anhydrous morphin) was introduced. Note, no depression.

ACTION ON THE BRONCHI

On examining the protocols of the various experiments already given, it will be seen that an idea of the effect on the dead space was obtained by an indirect method, subtracting the efficient ventilation from the total ventilation and dividing by the rate of respiration. These figures convey only approximately the effect of the drug on the bronchi. I have endeavored to ascertain the action on the bronchi further by the direct method of employing bronchial rings. This method first employed by the Italian observer, Titone (18), is in principle the same as that of studying arterial rings (Meyer) employed by the author in other connections (19). By this method it was found that morphin caused but slight broncho-dilatation, codein still less so, while narcotin and papaverin produced a more pronounced relaxation of the bronchial rings. It was furthermore interesting to note, that just as in the case of the coronary artery the actions of the morphin and narcotin tended to antagonize each other.

CONCLUSIONS

Summing up the action of the opium alkaloids on the respiratory function above described, we see that these drugs may be broadly divided into two classes. On the one hand is morphin, which is the great sedative alkaloid, although in small doses it may not interfere with efficient respiration, and may even improve it. On the other hand we have narcotin, papaverin, narcein, thebain, and cryptopin, which are all distinct stimulants and in large doses excitants of the respiratory center. Codein belongs to the morphin class, though in large doses it may also excite the respiratory center, especially in rabbits.

The rate of respiration is markedly slowed by morphin, the number being on the average diminished one-third by moderate doses of the drug. Codein also slows the respiration but to a much lesser degree. Narcotin, and papaverin decrease the rate but slightly; the other alkaloids not at all, and may even increase it.

The action of combinations of these alkaloids on the respiration can be completely explained as a summation of their individual properties. In this way it was found that morphin plus narcotin, morphin plus papaverin, and a mixture of the total opium alkaloids, is less depressant to the respiratory center than morphin alone. This was corroborated by Hooker's method of studying the effect on the center directly, by perfusing an animal's medulla, apart from the general circulation. On the bronchi narcotin and papaverin have a dilator action, morphin and codein also but to a lesser degree, the other alkaloids none at all, and furthermore the effects of morphin and narcotin seem to neutralize or antagonize each other.

SUMMARY

1. An improved and elaborate apparatus for studying respiration in intact unanesthetized animals has been described.

2. Of the primary opium alkaloids, morphin and to a lesser degree codein are sedative or depressant, while narcotin, papaverin, narcein, thebain, and cryptopin are stimulating to the respiratory center.

3. The action of combined opium alkaloids, is a summation of their individual effects.

4. On the bronchi, narcotin and papaverin are dilators, morphin is a dilator to a lesser degree, codein still less so, and the other alkaloids not at all. Morphin and narcotin seem to act antagonistically on the bronchi, their combined effect being a lesser dilatation than that produced by each of them individually.

5. It is hoped that the above findings will contribute to a more rational therapeutic use of opium and its derivatives.

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THE VASOMOTOR REACTION TO NICOTIN: LOCUS OF STIMULATION

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In connection with other researches we have felt the need of a more definite knowledge as to how nicotin produces its well known pressor effect. That the effect is due to sympathetic stimulation is generally recognized, but the exact locus of the excitation is uncertain. The textbook statement of the situation seems to be based largely upon a paper published by Langley and Dickinson 1890 (1). These investigators definitely proved that nicotin stimulates the sympathetic ganglion cells. They thought also that the vasoconstrictor centre in the medulla shares in the stimulation but considered that their researches did not determine the matter. Sollmann in his Textbook of Pharmacology definitely ascribes the pressor effect to central stimulation but does not cite convincing evidence (2). In a recent paper Pilcher and Sollmann have reaffirmed the central stimulating effect (3). Their evidence is that nicotin decreases the perfusion rate in an organ connected with the body only by its nerve supply. They did not, however, exclude the possibility that the stimulation occurred wholly or in part in the outlying ganglia. It is at present an open question to what extent the pressor effect may be due to an influence of the drug upon the vasoconstrictor centre proper, the spinal cord or the outlying ganglia.

We have investigated the problem in the following way: Nicotin in small standard doses was injected by vein and its pressor effect recorded. Then the spinal cord was cut at the eighth cervical level and the experiment repeated. Finally the

spinal cord was destroyed and the reaction to the standard dose again determined. To permit the detection of any changes in the condition of the peripheral tissues which might enter in to vitiate the experiments the effect of a constant dose of adrenin¹ was recorded from time to time. In most cases the vagi were cut and in some curare was used. In all, eighteen experiments were made.

Technique. All of the work was done with dogs under ether anesthesia. To avoid the entrance of shock and hemorrhage as complicating factors during the course of the experiments all operative procedures were carried out as far as possible in advance. The spinal cord was exposed, the dura mater incised and a strong linen ligature passed under it. This served as a guide in the subsequent resection of the cord. When the loop was liberated it also served to prove that the cord had been completely severed. Ordinarily the incision was closed and the animal allowed to recover from the anesthetic.

From two to four hours later the experiment was resumed. A tracheal cannula was first introduced to facilitate uniform etherization, during the remainder of the experiment. The vagi were isolated and cut. Blood pressure was recorded by means of an ordinary mercury manometer connected with a femoral artery. The reservoir cannula method previously described was used (4). Ten per cent sodium citrate served as anticoagulant. In quantitative drug experiments the rate of injection is an important factor. To secure uniformity in this respect the following procedure was used: A large bore venous cannula was tied in a femoral vein close to the inguinal ligament. This was connected with a reservoir of 0.8 per cent sodium chlorid solution at a height of two feet. The connecting tube was closed by a clamp close to the cannula. The drug to be given was injected into the tube close to the clamp which was then quickly released. The drug was thus instantaneously flushed into the vein. By this procedure the margin of experimental

¹ Following the usage of Schaefer, Vincent and Cannon, and hoping to aid in simplification of terminology we have adopted the term *adrenin* to indicate the active principle of the chromaffin tissue.

error can be reduced to negligible proportions and a series of closely similar reactions obtained from the same animal (4). In a few instances the animals were curarized and artificial respiration maintained by a Gesell and Erlanger apparatus (5). In the longer experiments hot water bags were used to keep up normal temperature.

The initial blood pressure having been recorded a moderate dose of "adrenalin" (usually 1 cc. of 1:100,000) was injected. In the earlier experiments nicotine was given in subminimal and gradually increasing quantities until the threshold value for pressor response was established. Also a larger standard dose sufficient to give a moderate rise of pressure was injected. Usually about 1 cc. of 1:4000 was required. Then while blood pressure was being recorded the spinal cord was cut at the eighth cervical level and the injection experiments repeated, the new threshold to nicotine being again determined as well as the reactions to the standard doses of adrenin and nicotine. The spinal cord was then destroyed and the injections again repeated.

To destroy the cord a piece of soft iron wire about 1.5 mm. in diameter was introduced through the cervical incision. The entering end was bent around to form an open loop, the distal limb of which was parallel to the rest of the wire. The rounded end facilitated introduction. It was thrust through the medullary canal with a rotary motion until well into the lumbar region. Upon withdrawal the open loop engaged the cord substance and completed the destruction. Examination at autopsy showed that the procedure left nothing to be desired in the way of thoroughness. The hemorrhage resulting from this procedure was comparatively slight in quantity, and seemingly played no part in results.

RESULTS

Cord transections. The outcome in most cases fulfilled previous expectations. If the experiments were carried through promptly without curare, transection of the cord ordinarily lowered the pressure from 30 to 50 per cent. The reaction to nicotine was materially depressed, usually about 50 per cent.

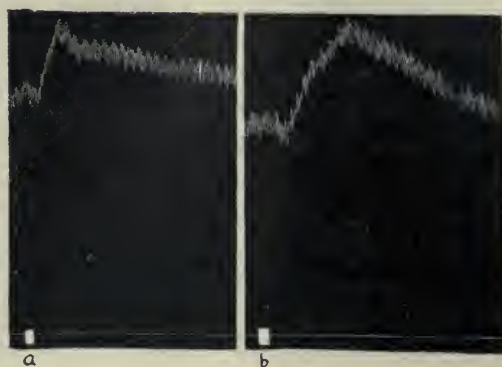


FIG. 1. Initial reactions to adrenin and nicotin. At *a*, intravenous injection. "Adrenalin" 1 cc. 1:100,000. At *b*, nicotin, 0.4 cc. 1:1000. Pressure from femoral artery. Base line, O-pressure and signals (reduced to $\frac{1}{2}$).



FIG. 2. Effect of cutting spinal cord, eighth cervical level (reduced to $\frac{1}{2}$).

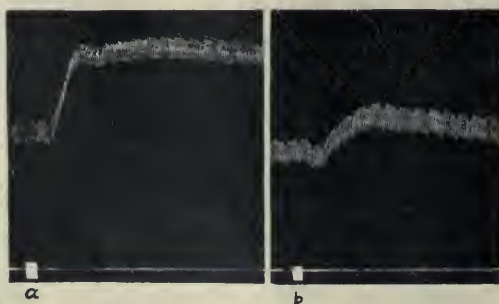


FIG. 3. Repetition of 1 after cord transection (reduced to $\frac{1}{2}$).

The adrenin reaction, on the other hand, was either unchanged or somewhat increased,—supposedly because of the lower blood pressure level.

Figures 1 to 5 show in order the significant features of one typical experiment. The dog was an adult of medium size (7 kilos). Figure 1 shows the reactions to the standard doses of adrenin and nicotin. Figure 2 shows the effect upon blood pressure of cutting the cord. The pressure fell from 120 to 65 mm. In figure 3 is seen the reactions to the standard doses of the drugs a few minutes after the transection. The nicotin pressor reaction was reduced from 48 to 22 mm. while the adrenalin reaction was slightly augmented,—from 40 to 46 mm. Figure 4

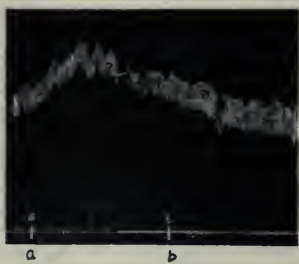


FIG. 4. Effect of destroying spinal cord. At *a* wire introduced. At *b*, withdrawn.

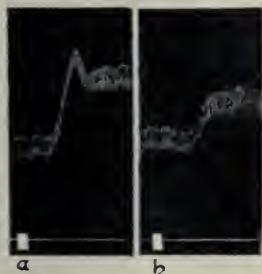


FIG. 5. Repetition of 3 after cord destruction (reduced to $\frac{1}{2}$). (Figs. 1-5, same animal.)

shows the effect of destroying the spinal cord through the thoracic and upper lumbar regions. The mean pressure fell rather less than usual, from 70 to 60 mm., while the reactions to nicotin and adrenin were, as seen in figure 5, essentially unchanged. Post-mortem examination showed that the transection had been complete and the cord destruction reached to the third or fourth lumbar vertebra.

In some instances interesting variations from these typical results were noted. Although the general blood pressure level and the reaction to nicotin were ordinarily depressed by cord transection in approximately equal degree, this was not al-

ways the case. Occasionally the general vascular tonus remained almost unchanged while the nicotin reaction was materially depressed. In one specific instance the general pressure was 116 mm. After the transection the pressure ran smoothly at a level of 112 mm. essentially the same as before. The reaction to 0.8 cc. of 1:4000 nicotin, however, was reduced from 40 to 18 mm. The reactions to adrenin were respectively 44 and 42 mm. The cord transection was verified post mortem. This observation shows that vascular tonus may be kept up practically to a normal height without the intervention of the medullary vasomotor center even when the center is capable of functioning. In a way this observation supports Porter's conclusion that the "vasotonic" center and "vasoreflex" center are different structures (6).

In two other cases closely similar results were observed, but in each instance the initial pressure was only 90 to 100 mm. The cord was destroyed in only one of these three cases. In that one the pressure immediately fell from 100 to 50 mm. These instances indicate that to a greater extent than is usually supposed vasomotor tonus may be mediated by the spinal cord itself.

In several of the earlier experiments the effect of the transection on the threshold value of the nicotin stimulation was determined. This was found to vary in a general way inversely as the height of reaction. Thus in a given case if the reaction was decreased 50 per cent about twice as much of the drug was required to give an initial rise. The procedure necessitated the use of a considerable quantity of nicotin, however, and unduly prolonged the experiment so that results toward the end were not fairly comparable with those in the beginning. In the later cases, therefore, the threshold determinations were omitted.

The results secured with curare were on the whole unsatisfactory. In the earlier experiments it was used in the hope that it would contribute to constancy in outcome but instead it entered as a disturbing factor. The nicotin reaction was not only immediately changed by its use but caused to vary at different stages of the experiment.

A noteworthy feature of the experiments was the change in the reaction picture after cord transection. Although the height of the nicotin reaction was decreased the persistence of the effect both in case of nicotin and of adrenin was typically augmented often to a striking degree (compare figures 1 and 3). The phenomenon in some cases might, if they stood alone, be ascribed to induce sluggishness in the circulation, but other instances were seen in which the persistence could not be accounted for on such grounds. Figure 6 illustrates the point. "a" shows the reaction to 1 cc. of adrenin before and "b" the reaction to the same dose after cutting the cervical cord. The initial pressure

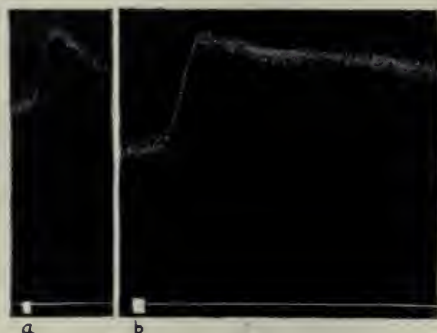


FIG. 6. Reaction to "adrenalin" 1 cc. 1:100,000; a, before, and b, after cord transection (reduced to $\frac{1}{2}$).

was only slightly lower after than before the transection, and the circulatory conditions during the reactions were practically identical. The reaction to nicotin in this experiment showed an exactly similar prolongation and need not be separately figured. The most plausible explanation of the persistence of reaction seems to be that cutting the cord eliminated a depressor mechanism that ordinarily comes quickly into play to damp the effect of pressor influences. Such an arrangement would have adaptive value in restoring normal circulatory conditions after momentary (accidental) stimulations.

Destruction of the cord. In most instances the destruction of the cord itself after cervical transection although it lowered the

vascular tension made no significant difference in the nicotin or the adrenalin reactions (compare figures 3 and 5). Occasionally, however, a slight depression of the nicotin curve was seen.

One other feature of the experiments seems worthy of mention. It might be expected that such a violent procedure as complete severance of the cord would cause a marked stimulation of the efferent vasomotor neurons and cause a sharp initial rise of pressure. In most cases, however, as in figure 2, nothing of the sort occurred. In no case was more than a very moderate rise observed.

Discussion. To what extent is *spinal shock* a factor in such experiments as these reported? The transection causes a marked depression in the spinal cord aboral to the level of the cut. This depression has been supposed to be due to an initial violent stimulation of inhibitory fibers which causes a more or less complete, but also a more or less temporary, suspension of the spinal reflexes. Sherrington (7) and Pike (8) reject this explanation, since the same depression can be produced by freezing the cord, a process which could not stimulate inhibitory fibers. Both of the authors, just mentioned, agree that there occurs in spinal shock an interruption in the spinal reflex arcs, which prevents the transfer of impulses from afferent to efferent neurones. They differ fundamentally as to the nature of this interruption, but this does not concern us here. The point of importance is that spinal shock is not due to a *depression of efferent neurones*, following stimulation of inhibitory fibers, but to a *break in the connection* between afferent and efferent elements. If, therefore, as is generally supposed, nicotin acts as a direct chemical stimulant to the efferent neurones, its action would not be affected by spinal shock.

SUMMARY AND CONCLUSION

Experiments on 18 dogs have shown that:

(1) Cutting the spinal cord at the eighth cervical level typically reduces the pressure reaction to nicotin about 50 per cent. The amount of nicotin necessary to produce an initial rise is approximately doubled.

(2) Further destruction of the cord through the dorsal and upper lumbar regions only occasionally induces a further depression in the reaction.

(3) The adrenin reaction is not significantly decreased.

(4) The pressor effect of nicotin is due about half to a stimulation of the vasoconstrictor (vasoreflex?) center proper and half to a stimulation of the ganglion cells. Occasionally spinal cord stimulation also contributes slightly to the reaction.

(5) In such experiments curare is not only useless but is a disturbing factor.

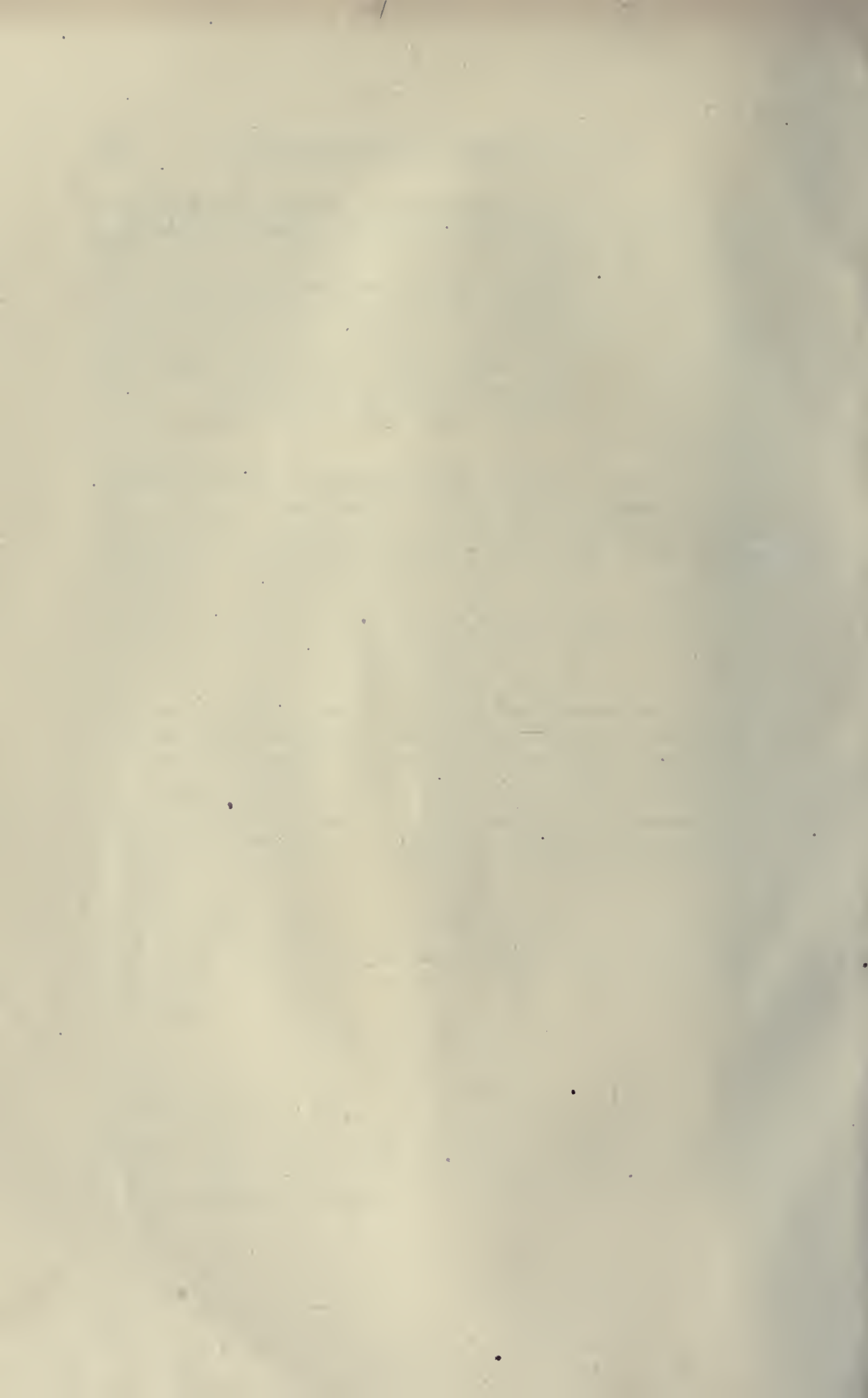
(6) Vasomotor tonus in some instances is largely mediated by the spinal cord independent of the medulla.

(7) Cutting the cord prolongs the pressor effect both of nicotin and adrenin, supposedly by eliminating a depressor mechanism.

(8) Spinal shock is probably not a factor in the results described.

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THE SIMILARITY AND SYNERGY OF MORPHINE AND STRYCHNINE ACTION

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It is well known that adequate doses of morphine, like strychnine, will produce tetanic convulsions in frogs. The action of morphine on mammals is variable. In cats and some other animals, morphine in sufficient amount will produce great excitement and sometimes spasms. In the dog and most mammals it is commonly taught that convulsions can be produced only after the administration of comparatively large doses and when artificial respiration is used to keep the animal alive. We have recently shown that tetanus follows very small doses in the dog under some conditions.¹ The great difference in the action of morphine in animals is usually explained on the basis of differences in the development of their nervous systems. In the most highly differentiated systems only the quieting and sedative action is seen, while in those animals with little brain development—such as the frog—the cord action is most pronounced. The results of our work lead us to doubt these explanations.

EXPERIMENTAL WORK

I. THE ACTION OF MORPHINE AND STRYCHNINE ON FROGS

In order to determine whether or not there is any relation between the action of morphine and strychnine on frogs, a number of experiments were made in which these alkaloids were injected under various conditions and the time of the onset of tetanus observed. The injections were all made into the anterior lymph

¹ McGuigan and Ross: *Journal American Medical Assoc.*, 1915, lxiv, 1494.

sac and the same amount of fluid used in all comparisons. Usually the volume injected was 1 cc.

I. November 1, 1912. Strychnine only.

<i>Weight of Frog</i>	<i>Amt. Strychnine mgm.</i>	<i>Onset of Tetanus minutes</i>
21	$\frac{1}{20}$	18
35	$\frac{1}{20}$	26
61	$\frac{1}{20}$	38
61	$\frac{1}{20}$	20
Average weight, 47 grams.		Average time of tetanus onset, 25.5 min.

II. Morphine followed by strychnine:

In this series the morphine was injected $3\frac{1}{2}$ hours before the administration of the strychnine. Amount of morphine injected—1 cc. 4 per cent morphine sulphate.

<i>Weight of Animal</i>	<i>Amt. Strychnine mgm.</i>	<i>Onset of Tetanus minutes</i>
60	$\frac{1}{20}$	13
60	$\frac{1}{20}$	14
52	$\frac{1}{20}$	12
Average weight, 57 grams.		Average time of tetanus, 13 min.

There was no indication of tetanus from the action of morphine alone although tetanus would probably have developed later had no strychnine been given. That the spasm recorded here was due probably to morphine is precluded by later experiments.

III. October 6, 1912. Strychnine only.

<i>Weight of Animal</i>	<i>Amt. Strychnine Sulphate mgm.</i>	<i>Onset of Tetanus minutes</i>
40	$\frac{1}{4}$	4
54	$\frac{1}{4}$	8
56	$\frac{1}{4}$	4
46	$\frac{1}{4}$	6
Average weight, 49 grams.		Average time, 5.5 min.

IV. In this case 1 cc. morphine sulphate was given immediately preceding the injection of strychnine— $\frac{1}{4}$ milligram.

<i>Weight of Animal</i>	<i>Amt. Strychnine Sulphate mgm.</i>	<i>Onset of Tetanus minutes</i>
48	$\frac{1}{4}$	5.5
40	$\frac{1}{4}$	4.5
49	$\frac{1}{4}$	5.0
48	$\frac{1}{4}$	6.0
Average weight, 46 grams.		Average time, 5.25 min.

The amount of strychnine used in this case does not give time for the best observations but the results are the same as with smaller doses and when Spring animals are used as shown by experiments V and VI.

V. *April 15, 1915.* Strychnine only.

<i>Weight of Animal</i>	<i>Amt. Strychnine mgm.</i>	<i>Onset of Tetanus minutes</i>
48	$\frac{1}{10}$	8
48.5	$\frac{1}{10}$	17
48	$\frac{1}{10}$	18
51	$\frac{1}{10}$	15
Average weight, 48.8 grams.		Average time, 14.5 min.

VI. Morphine and strychnine together: morphine 1 cc. 5 per cent.
Action of morphine when given 30 minutes before the strychnine:

<i>Weight of Animal</i>	<i>Amt. Strychnine mgm.</i>	<i>Onset of Tetanus minutes</i>
50	$\frac{1}{10}$	18
51	$\frac{1}{10}$	15
49	$\frac{1}{10}$	19
50	$\frac{1}{10}$	15
Average weight, 50 grams.		Average time of spasm, 16.75 min.

VII. 1 cc. of 5 per cent morphine was given 30 minutes before strychnine:

<i>Weight of Animal</i>	<i>Amt. Strychnine mgm.</i>	<i>Onset of Tetanus minutes</i>
45	$\frac{1}{10}$	10
56	$\frac{1}{10}$	9
45	$\frac{1}{10}$	8
49	$\frac{1}{10}$	9.5
Average weight, 49 grams.		Average time of tetanus, 9.1 min.

VIII. In this experiment 1 cc. 5 per cent morphine was given 107 minutes before the strychnine.

<i>Weight of Animal</i>	<i>Amt. Strychnine mgm.</i>	<i>Onset of Tetanus minutes</i>
47	$\frac{1}{10}$	8
52	$\frac{1}{10}$	9
49	$\frac{1}{10}$	5
47	$\frac{1}{10}$	7
Average weight, 49 grams.		Average time of tetanus, 7.25 min.

This is about one-half the time in which the strychnine alone would have caused tetanus.

Very large doses of morphine still produce the acceleration of the strychnine action but the tetanus in this case is followed quickly by paralysis and by fibrillation of the thigh muscles especially. In test IX a series of four animals weighing 60, 71, 72 and 77 grams were given 1 cc. of 10 per cent morphine sulphate two hours before $\frac{1}{10}$ mgm. of strychnine sulphate. Tetanus developed in 6 minutes, but there was only one twitch followed by paralysis of the flexors. Extension of the leg muscles seemed little influenced. There was marked fibrillation of the thigh muscles. This fibrillation develops immediately in many cases of strychnine tetanus if a dose of morphine be given after the development of the spasm.

To summarize the results on frogs, the following table of averages is compiled from the foregoing figures:

TABLE 1.
Average results of morphine and strychnine injections in frogs

TEST NO.	NO. OF FROGS USED	WEIGHT AVERAGE	MORPHINE USED	AMOUNT STRYCHNINE	TIME AFTER MORPHINE WHEN STRYCHNINE GIVEN	ONSET OF SPASMS
		<i>grams</i>	<i>mgm.</i>	<i>mgm.</i>		<i>minutes</i>
I.....	4	47	none	0.05	210	25.50
II.....	3	57	40	0.05		13.00
III.....	5	19	none	0.25		5.50
IV.....	4	46	50	0.25		5.25
V.....	4	48.8	none	0.10	30	14.50
VI.....	4	50	50	0.10		16.75
VII.....	4	49	50	0.10		9.10
VIII.....	4	49	50	0.10		7.25
IX.....	4	70	100	0.10	120	6.00

Tests I and II indicate that a previous injection of morphine hastens the action of strychnine. However the time intervening between the injection of the morphine and the injection of the strychnine is an important factor as is shown in tests from III to IX. When both alkaloids are injected at the same time or close together morphine seems to have no effect in hastening the onset of tetanus. When the time between the injections varied from

30 to 120 minutes the time of the onset of the tetanus decreases from 16.75 to 6 minutes.

These results indicate that the tetanus produced by morphine is not due to the action of the alkaloid itself but to a decomposition product of it. The change is probably due to oxidation of the morphine by the tissues and tetanus from the morphine probably develops when a sufficient amount of the tetanic product has accumulated, provided it can reach the nerve centers. To test this conjecture we have carried out a series of oxidation tests which seem to verify it.

April 19, 1915. To show that oxidation changes the morphine into a substance having a strychnine like action the following experiments were made: Solutions of 10 per cent morphine were treated as follows:

- 1..... 5 cc. 10 per cent morphine plus 0.1 cc. strong HNO_3
- 2..... 5 cc. 10 per cent morphine plus 0.2 cc. strong HNO_3
- 3..... 5 cc. 10 per cent morphine plus 0.5 cc. strong HNO_3
- 4..... 5 cc. 10 per cent morphine plus 2.0 cc. strong HNO_3
- 5..... 5 cc. 10 per cent morphine plus 1.0 cc. strong HNO_3

These mixtures were allowed to stand over night and then neutralized carefully with sodium bicarbonate. The natural color of the solutions serves as indicator. After neutralization each of the solutions was made to 5 per cent morphine (original).

The action of the oxidized morphine 5 per cent was then compared with the action of a similar amount of unoxidized morphine on frogs and dogs.

Freshly prepared: morphine, action on frogs:

Test X. Controls.

<i>Weight of Frog</i>	<i>Morphine 5 per cent cc.</i>	<i>Onset of Spasms minutes</i>
27	1	60
29	1	61
27	1	67
25	1	78
30	1	50
30	1	58
36	1	60
33	1	100
Average weight, 29 grams.		Average time, $66\frac{3}{4}$ min.

Test XI. Results from the injection of solution No. 1 which is the action of 0.1 cc. HNO_3 on 5 cc. 10 per cent morphine for about 18 hours.

Weight of Frog	Morphine 5 per cent cc.	Onset of Spasms minutes
38	1	70
48	1	72
27	1	62
30	1	70
Average weight, 36 grams.		Average time, 71 min.

Test XII. Solution No. II. The action of 0.2 cc. HNO_3 on 5 cc. 10 per cent morphine solution for about 18 hours at room temperature.

Weight of Frog	Morphine Equivalent to 5 per cent cc.	Onset of Spasms minutes
30	1	45
25	1	31
28	1	39
28	1	38
Average weight, 28 grams.		Average time 38 min.

Test XIII. Solution No. III. Due to the action of 1 cc. HNO_3 on 5 cc. 10 per cent morphine, and finally made to 10 cc. = 5 per cent morphine.

Weight of Frog	Morphine Equivalent to 5 per cent cc.	Onset of spasms minutes
30	1	5
II..... 30	1	5
III.....	1	7
IV.....	1	5
V.....	ms.	Average time, 5½ min.
VI.....		
VII.....		
VIII.....		
IX.....	4 No. 4. Due to the action of 2 cc. HNO ₃ on 5 cc. 4 phosphate. 4	
	Morphine Equivalent to 5 per cent cc.	Onset of Tetanus minutes

Tests I and II indicate that strychnine hastens the action of strychnine is an important factor between the injection

IX. When both alkaloid solutions were neutralised and diluted together morphine seems to be more potent. The concentration was considerable and undoubtedly of tetanus. When the time of onset was considered. This is easily seen in the salt fibrilla-

tion that is present in many cases. However, the salt is not the principal cause of the change in the time of the onset of the tetanus as is seen in the case of the mammalian experiments and in the following controls with the frogs: Tests were made in which the morphine was used alone, second morphine sulphate plus salt (solution 5) and third oxidized morphine sulphate—solution 3 were used. The average weight of the frogs was about 15 grams. Each frog was given 1 cc. of the solution. The following table gives the results:

XV. Time of the onset of spasms in minutes.

<i>Frog</i>	<i>10 percent Morphine Sulphate minutes</i>	<i>Solution 5 minutes</i>	<i>Solution 3 minutes</i>
1	53	20	13
2	52	73	23
3	36	37	16
	Average, 47	43.3	17.3

These averages are borne out by other experiments and are confirmed in a striking manner by the results on mammals.

TABLE 2
Average results of tests with oxidized morphine

TEST	MORPHINE SULPHATE	SOLUTION I	SOLUTION II	SOLUTION III	SOLUTION IV	SOLUTION V
X.....	66.75	0.71	38	5.5	12.75	43.3
XI.....						
XII.....						
XIII.....				5.5		
XIV.....					12.75	
XV.....	47			17.3		

Tests X, XI, XII, and XIII indicate that morphine may be oxidised to a certain degree where the tetanizing activity of the product is maximal. Test XIV indicates that this oxidation product may be further oxidised to a less active substance. The action of the salt as shown in XV has some influence on the development of the tetanus but it cannot be responsible for any large amount of the time reduction in the onset of the spasm.

It seems therefore that when morphine is injected into frogs it is slowly oxidised to a substance which will produce tetanus and as seen in the case of dogs the oxidised product is quite distinct from the ordinary action of morphine. The tetanus produced is similar in type to that caused by strychnine and there is no reason for thinking that the mechanism is different. In many cases the oxidised morphine causes a distinct picrotoxin like action which finally gives way to the strychnine type of spasm. This we think is not so much a difference in the manner of action as it is to a difference in place of action. The medulla seems first to be influenced in these cases.

II. THE ACTION OF MORPHINE WHEN INJECTED INTRASPINALLY IN MAMMALS

That morphine causes excitation in some mammals is well known; but to obtain even a moderate tetanus in dogs very large doses of morphine must be given and artificial respiration used to keep the animal alive. The action of morphine in the frog suggests the possibility of a similar action in dogs if the action on the respiratory center could be avoided. McGuigan and Becht² have shown that strychnine injected into the cerebrospinal fluid in any region elicits spasms that are confined to the corresponding segment for a considerable period of time. It was thought, therefore, that because of the similarity of the cord action of strychnine and morphine, injection of the latter into the lumbar region should give the morphine action in the corresponding segment some time before it penetrated to the respiratory center. This was found to be the case, and it was also found that injection into the fourth ventricle gave typical strychnine-like spasms without much interference with the respiration.

(a) *Method of injection of drugs into the cerebrospinal fluid*

In our earlier work the injections were made under light ether anesthesia. In all later experiments no anesthetic was used.

² McGuigan and Becht: Jour. of Pharm. and Exper. Ther., 1914, vol. v, p. 469.

The animal was tied on the operating board and the head bent forwards; the injecting needle was inserted about the junction of the first and middle thirds of the distance between the occiput and first cervical vertebra, the point of the needle pointing slightly backward. An amount of the fluid was withdrawn—the same volume as the injection made. There is very little pain connected with the operation or injection. All solutions injected were neutral in reaction.

(b) *Injection of small doses of morphine sulphate in region of fourth ventricle*

Test 1. In an animal 9 kilos 1 cc. of 0.01 per cent morphine sulphate produced about the same action as a moderate dose of morphine subcutaneously except that there was no vomiting. There was some salivation and the lack of vomiting was perhaps due to the previous ether anesthesia.

Test 2. Still another animal—4 kilos—was given $\frac{3}{4}$ cc. of 0.1 per cent morphine into region of the fourth ventricle without anesthesia. This produced vomiting and purging followed by the normal effect of morphine.

Test 3. One-tenth of this dose, $\frac{3}{4}$ cc. of a 0.01 per cent in a 7 kilo animal was almost without effect. There was a slight depression. No vomiting or purging.

Test 4. Injection of large dose in cord above ilium.

Dog 12 kilos. Anesthetised with ether.

9.25. 1.5 cc. 10 per cent morphine sulphate injected into cord just above the ilium.

9.40. Exceedingly sensitive about the tail. Knee jerks are easily elicited and are increased. Stimulus over point of injection shows marked increase in sensitivity. There is evidence that the action is spreading upward. This perhaps is due to the large volume of the injected fluid.

10.02. Knee jerk becomes more like spasm. Stimulation over the shoulder girdle gives response in the hind legs, very slight in front.

10.05. Stimulation of the knee gives three kicks or responses. The most sensitive points are around root of tail and the internal canthus of the eye.

10.15. Tapping the tendon of the front leg elicits no response of the

front leg but elicits kicks of both hind legs. There is a marked difference in the response of the front and hind legs.

10.20. Stimulation of the sole of the hind foot produces tetanus of hind legs alone. The same stimulation of the front legs gives no response of the front legs while it may elicit spasm of the hind legs.

10.40. Stimulation over the hind leg area gives tetanus of the hind legs—not transmitted to the front. Stimulation over the ribs gives no response. There seems to be a zone between the hind leg area and the shoulder girdle that is less sensitive than either of these regions. There is a condensed saddle like area—like the scratch reflex area of Sherrington—which is extremely sensitive, stimulation of which, elicits responses in both front and hind legs—more pronounced in the hind legs. The transmission is more caudalward than towards the head, and the reaction to the stimulus more below the stimulus than directly over it.

10.50. Stimulation of the front feet easily elicits a spasm of the hind legs but the reverse is not the case. Just now stimulation of the hind feet may cause an extension of the front legs. There is a decided pilomotor action along the spine. Respiration has been regular throughout.

11.10. Stimulation of the hind foot causes extension of the hind legs.

12.15. Spontaneous and continuous tetanus—hind legs more involved than the front.

1.30. Animal appears mentally normal. The hind legs are tetanic—contracture. Front legs are extended when the animal is moved.

3.30. Animal in general tetanus, neck muscles involved and the head bent on the chest—the opposite of strychnine. The action is much like tetanus toxin. Hind legs in strong tetanus. Heart beat 36 per minute, respiration slow. At this time it seems that the animal will die from the action of the drug.

March 18. Noon. Animal apparently recovering. Hind legs somewhat paralyzed. Animal walks with difficulty; is stuporous but apparently will recover.

Five days afterward the animal had completely recovered and was used for another experiment.

(c) Injection morphine-sulphate into region of fourth ventricle

Test 5. Red terrier, 6 kilos. Lightly etherized.

9.45. 1 cc. 0.3 per cent morphine sulphate in the region of the fourth ventricle and the ether removed.

10.00. Dog sleeps quietly.

11.00. Little change. Decided morphine coma. Slight increases in the reflexes of hind legs.

1.00. Little change. Involuntaries—rectal and vesical.

3.00. A marked flow of saliva, otherwise animal in same condition as previously. The animal was placed in the cage and was found dead next morning.

Test 6. Dog 5 kilos. Female collie, spirited animal.

9.20. $\frac{3}{4}$ cc. 1 per cent morphine sulphate injected into the fourth ventricle under light ether anesthesia.

9.30. Pronounced scratching of head and ears with both front and hind legs. Yelps and whines considerably.

9.45. Violent scratching movements directed to head. Nose held between front legs.

10.00. Quiet and sleeping.

11.00. Very excitable—in position of pleurothotonous. Reflexes very active.

11.20. Runs about excitedly—growling with tongue hanging out. Delirious.

11.25. Rabid—extreme case—froths at mouth and bites at anything in the way.

11.45. Same condition. Yelps and howls considerably. Eyes dilated and protruding. Salivation pronounced. Ears twitching and some air hunger. Extreme pilomotor effect wider over shoulders.

12.15. Quiet—but a continuous twitching and jerking of the legs—highly irritable. Partial paralysis especially of the flexors (cf. frog under large doses of morphine). Twitchings almost tetanic.

12.30. Spasmodic continuous jerking, with exaggerated periods like tetanus; these periods are becoming more frequent.

12.44. Spasm.

12.46. Spasm resembling strychnine.

1.00. Quiet.

1.15. Quiet and reflexes greatly decreased. The animal continues in this state becoming sleepy, finally unconscious and continues to fail until there is a cessation of all functions.

2.55. The dilation of the pupils, pilomotor action and the action of the intestines in all cases, indicates an action on the sympathetic nervous system.³

³ Mostrom and McGuigan: Jour. of Pharm. and Exper. Ther., 1912, iii, p. 515, have shown that epinephrine and strychnine have some synergistic actions.

Test 7. Female fox terrier, 6 kilos. Dog I.

9.35. Given 0.5 cc. 3 per cent morphine sulphate in region of fourth ventricle under light ether anesthesia.

9.50. Slight twitching and jerking of the legs. That of the hind legs a fraction of a second later than the front. The jerks are rhythmic and at the rate of 200 per minute.

9.55. Any slight stimulus starts a scratching movement of all four legs and a crossing of the front legs. Pupils widely dilated. Rhythmic twitchings continue at the same rate.

10.00. Scratching movements continue. Animal delirious. Considerable incoördination and yelping. Dog wild—apparently rabid. Heart 180 per minute. Thick foam from mouth. Not profuse. Legs stiff, claws contracted. Partial paralysis of flexors of hind legs—pupils dilated.

10.30. Extreme pilomotor effect. Respiration jerky and asphyxial. General spasm.

11.16. Spasm has been repeated about every five minutes and now the animal dies in a strong spasm. The spasm commenced with a fine tremor or twitching around the nostrils and swallowing like movements. Perhaps a slight increase in the saliva. Head bends forward on the chest. Front legs extended, stiff and directed toward the abdomen. Hind legs in stiff scratching movements on the abdomen, back curved with tail to abdomen. There may be a gradual change to pleurothotonus revolving into a distinct strychnine like episthotonus with both head and legs extended. After a few relaxations and extensions the animal died. In this case it could not be determined whether heart or respiration stopped first.

Test 8. Dog, 10 kilos. Red mongrel setter.

8.15. 1.5 cc. 5 per cent morphine sulphate injected into the middle of the cervical cord, under light ether anesthesia.

9.00. Reflexes increased. Tendency to scratch neck region of injection. Front legs crossed. Pupils dilated. Cyanotic. Salivation and excitement.

9.05. Great tension of neck muscles. Some stiffness of thigh muscles, and front legs. Tail stiff. Air hunger. Cannot stand up. Strong general convulsions.

9.10. Heart beat 180. Thick mucous from mouth.

9.20. Strong convulsions. Front legs stiff and extended. Hind legs in a continuous swimming or scratching movement. Head extended and neck muscles rigid. Front legs extended.

9.25. Cheyne-Stokes respiration. Asphyxiation is due to the spasms. Heart irregular.

10.04. After repeated spasms respiration stops perceptibly before the heart. The experiment is similar to the others. Strong action at the point of the injection and scratching movements directed to the point of injection.

Test 9. Fox terrier, weight 10 kilos.

10.15. Animal etherized, 1.5 cc. 5 per cent morphine sulphate injected into fourth ventricle and ether removed.

10.30. Animal sleeps.

10.40. Scratches at head and nose with all four feet as if to remove some annoying stimulus or irritation. This movement has been observed in all animals so treated. The head end of the animal is much more influenced than the tail end. The front legs are crossed and stiff. The pupils dilated.

10.45. Spasms resembling strychnine—especially in the front legs. Pupils widely dilated—not asphyxial.

10.46. Strong spasm—head back, the body arched as in defecation, hind legs in a swimming like or scratching movement. Some air hunger.

10.50. Spasm repeated. Heart 216 per minute.

10.52. Violent spasm—animal yelps occasionally.

10.54. Snorts—asthmatic like—pronounced pilomotor effect along the back.

11.10. Froths at mouth—asphyxial respiration—seems mainly expiratory. Ether relaxes animal.

11.14. 5 cc. 5 per cent morphine intravenously increases spasm.

11.45. Animal dies in spasm. Respiration stops before the heart. Considerable foaming at the mouth. The action throughout very much like strychnine. The manner of exit was different here from that of the previous animal.

(d) Injection of the oxidized product into mammals

The oxidized product when injected intravenously into dogs seems to have little effect. An amount of solution III was injected into a healthy dog 7 kilos as follows:

Test 10.

10.15. 1 cc. 5 per cent intravenously—into femoral.

10.30. No indication of any action whatever.

- 10.35. 3 cc. more injected as before.
10.37. Some vomiting and defecation—of mild grade.
11.00. Animal appears normal.
1.00. Practically normal—no morphine action.

Test 11.

2.00. Dog weighing 3.3 kilos was given intraperitoneally 12.5 cc. of the oxidized morphine.

- 2.07. Vomited.
3.00. Vomited several times. Salivated. Uneasy.
4.00. Normal.

Next day, normal.

Test 12. II. A healthy dog, 15 kilos, was given 1 cc. of solution No. 4 equivalent to 1 cc. 5 per cent of the original morphine solution, into the region of the fourth ventricle. The animal was in typical strychnine like tetanus instantly and was dead in 20 minutes after showing a series of the principal actions as indicated in the prolonged cases of pure morphine.

Test 13. Dog No. III. Weight 7 kilos.

Solution No. 4 was diluted with water to represent 1 per cent of the original morphine.

2.02. $\frac{1}{2}$ cc. was injected into the fourth ventricle and instantly the animal was in a strychnine like convulsion. Stiffness of all muscles very pronounced without much tetanic action or shaking.

2.08. Animal vomits.

2.15. Very strong tetanus followed by relaxation and running like and swimming like movements. Opisthotonus, air hunger, salivation—thick mucous like. Typical spasms repeated about every minute until 2.35 when the animal died. The action was little different from those given in detail above, except that it started instantly on the injection of the oxidized product.

2 grams of morphine sulphate were boiled with 40 cc. N/2 HNO_3 for twenty minutes. When the solution had evaporated to 18 cc. it immediately turned red and some of the oxids of N. were given off. 20 cc. of water were added and the whole again evaporated to 20 cc. over a free flame, and then neutralized with sodium bicarbonate. This was then diluted so that it contained 0.5 per cent of the original morphine sulphate. The saline content—determined by evaporation to dryness and subtracting the supposed morphine content, was 0.2 per cent. Total solid content was 0.7 per cent. This was used as the oxidized morphine in the following work on cats.

Test 14. Cat No. I, 2.7 kilos. 0.5 cc. of this morphine solution were injected in the same manner as in the case of the dogs—ether had to be used in this case.

11.35. Injection 0.5 cc. in region of fourth ventricle.

11.40. Scratches head with fore feet and becomes rabid.

11.43. Terrific spasms. Foams at mouth, pupils widely dilated and extreme pilomotor action. Opisthotonus very pronounced. Animal etherized and experiment ended.

Test 15. Cat No. II, 3 kilos. No ether was used on this animal, and freshly prepared unoxidized morphine 0.55 per cent in water was used.

11.25. 0.5 cc. injected into region of fourth ventricle. Pupils dilate almost immediately. In ten minutes there was a little excitement, perhaps due to disturbance of vision. No other action, and no indication of wildness or tetanus. After two hours the animal was practically normal and at no time showed excitement except when artificially stimulated. The animal completely recovered.

(e) *Injection of drugs related to morphine and their oxidation products*

If, as indicated in the recorded experiments, the convulsant action of morphine is due to an oxidised product of morphine, we should expect the same action when related bodies are oxidised.

The oxidation was carried out as follows: 4 cc. of 1 per cent codeine sulphate solution was mixed with 1 cc. of N/1 HNO_3 , and the mixture evaporated to dryness on an electric plate at low heat. A little water was added and the mixture again heated. This was carefully neutralized with sodium bicarbonate and the solution evaporated to 8 cc.. This corresponds to a 1 per cent solution. 4 cc. of this was used to determine the total solid content which was found to be 1.325 per cent. If the codeine was 1 per cent this left a saline content of 0.325 per cent. Even if the total solid were NaNO_3 it would not have produced the action recorded below.

Test 16.

11.05. 2 cc. of 1 per cent codeine sulphate was injected into the region of the fourth ventricle of a young dog of 4.5 kilos after the withdrawal of 2 cc. cerebro-spinal fluid.

11.10. Rests quietly.

1.30. In same condition, no evidence of convulsions.

Test 17.

1.33. 2 cc. cerebro-spinal fluid withdrawn and 2 cc. of a 1 per cent solution of oxidized codeine sulphate injected.

1.35. Animal commenced to scratch head as after the injection of morphine.

1.40. Pronounced scratching.

1.44. Spasm.

1.50. Strong spasm—experiment ended.

It was found that codeine, apocodeine and apomorphine when injected into the region of the fourth ventricle did not produce convulsions. It would seem therefore that these substances are not oxidised in the body. The oxidised products of all these bodies however produce convulsions. The above experiment with codeine may be taken as illustrating the action of all these drugs.

(f). Control injections to eliminate questions of the effect of added factors.

In all oxidations with HNO_3 as recorded above there is produced some of the oxids of nitrogen and some salt which might be blamed for the physiologic reaction of the oxidised drug. To eliminate this factor we made a control where cane sugar was oxidised with the nitric acid in the same manner as the codeine and the morphine.

Test 17. A dog weighing 10 kilos was given by way of fourth ventricle 2 cc. of the solution (containing 0.05 gram sugar and salt resulting from the neutralization of 1 cc. N/1 HNO_3 with NaHCO_3).

Result. No effect.

There was still some question as to whether the action of the oxidised drugs was not due to a slight excess of alkali or acid in the final solutions injected. To "control" this considerable quantities of acid and alkali (much more than could have occurred in our solutions) were injected as usual. No convulsive effects appeared.

The amount of salts formed in the solution of oxidised alkaloids was determined. Doses of salts much larger than these were injected without producing any excitement.

TABLE 3
Summary of results on mammals

TEST NO.	ANIMAL	WT. ANIMAL IN KILOS	ALKALOID INJECTED	VOLUME INJECTED	WT. OF ALKALOID IN MGMS.	POINT OF INJECTION	ONSET OF SPASM IN MINUTES
1.....	Dog	9.0	Morphine sulphate	1.00	0.100	4th ventricle	None
2.....	Dog	4.0	Morphine sulphate	0.75	0.750	4th ventricle	None
3.....	Dog	7.0	Morphine sulphate	0.75	0.075	4th ventricle	None
4.....	Dog	12.0	Morphine sulphate	1.50	150.000	Cord above ilium	General 365
5.....	Dog	6.0	Morphine sulphate	1.00	3.000	4th ventricle	None
6.....	Dog	5.0	Morphine sulphate	0.75	7.500	4th ventricle	264
7.....	Dog	6.0	Morphine sulphate	0.50	15.000	4th ventricle	55
8.....	Dog	10.0	Morphine sulphate	1.50	75.000	Below 4th ventricle	50
9.....	Dog	10.0	Morphine sulphate	1.50	75.000	4th ventricle	30
10.....	Dog	7.0	Oxidized morphine	1.00	50.000	Femoral vein	None
11.....	Dog	3.3	Oxidized morphine	12.50	500.000	Intraperitoneally	None
12.....	Dog	15.0	Oxidized morphine	1.00	50.000	4th ventricle	Immediately
13.....	Dog	7.0	Oxidized morphine	0.50	5.000	4th ventricle	Immediately
14.....	Cat	2.7	Oxidized morphine	0.50	2.500	4th ventricle	Immediately
15.....	Cat	3.0	Morphine sulphate	0.50	2.500	4th ventricle	None
16.....	Dog	4.5	Codeine sulphate	2.00	20.000	4th ventricle	None
17.....	Dog	4.5	Codeine sulphate oxidized	2.00	20.000	4th ventricle	11
18.....	Dog	10.0	Oxidized cane sugar	2.00	50.000	4th ventricle	None

* Apomorphine and apocodeine act in the same way as codeine.

SUMMARY AND CONCLUSIONS

The results on table 1 show first, that if morphine be injected some time before strychnine it will hasten the onset of tetanus in frogs. Second, the longer the time interval between the injection of morphine and the subsequent injection of strychnine, the greater will be the decrease in the time necessary for the onset of strychnine-like tetanus. It is well known that in most animals adequate doses of morphine will cause depression rather than stimulation of the nervous system. To harmonize such discordant results with this and other drugs on various animals several assumptions have been made.

First. Morphine requires some time to penetrate to and to paralyze the inhibitory parts of the brain which are connected with the coördinating centers; hence convulsions appear only

after the period necessary for such penetration and paralysis. Such an assumption was made by Barbour and Abel⁴ to explain the convulsions produced by acid fuchsin and accepted with slight modification by Meltzer and Joseph⁵ for the same substance and also for morphine. When the controlling centers are paralyzed or removed, less morphine is required to produce convulsions.

Second. The degree of development of the nervous system of the frog may be so different from that of the more highly organized system of mammals that convulsive drugs act in an entirely different way.

Third. It is not morphine *per se* but its oxidised products that are convulsive. The rate and extent of metabolism in frogs and especially their power to oxidise morphine is so much less than in mammals⁶ that the convulsive intermediate products we think have a longer existence in the body and hence a better opportunity to act on the nervous centers. In mammals the oxidation is so much more rapid that the convulsive products apparently have a very limited existence and hence no time to accumulate and produce such action.

If the first assumption were true for morphine, oxidation should not decrease the latent period but since it does, the hypothesis is not sufficiently comprehensive. It may however be a factor of more importance in some other drugs than it is in the case of morphine. It is quite evident also that the sedative action and the convulsant action of morphine are due to entirely different chemical entities. The sedative action is elicited almost immediately after a hypodermic injection; but only after a long latent period does the convulsant action develop. After artificial oxidation the sedative action is lost while the convulsant action which remains in certain cases is increased, further oxidation destroys the convulsant action also. We know nothing of the nature of this convulsant body; it can not be extracted by ether or chloroform.

In the second assumption we are prone to lose sight of the fact

⁴ Barbour and Abel: Jour. of Pharm. and Exper. Ther., 1910, vol. ii, p. 165.

⁵ Meltzer and Joseph: Jour. of Pharm. and Exper. Ther., 1911, iii, 183.

⁶ Frankel: Archiv für experimentelle Pathologie und Pharmacologie, 1910, lxiii, 331.

that the nervous system of the lower animals differs from that of the higher in quantity rather than in quality. The physico-chemical property that brings about stimulation or depression in the two must be the same; otherwise how can we justify much experimental pharmacology? Therefore we are led to discard the second assumption.

The third assumption is based on the fact that the general metabolism and oxidation in amphibians is much slower than in mammals⁷ which suggests that oxidation may play an important part in explaining the differences in the action of morphine in the two classes of animals. Cloetta⁸ and Faust⁹ have shown definitely that morphine is oxidised in the body and Frenkel¹⁰ has given reason to believe that it is oxidised in the brain and cord and that the oxidation is much slower in frogs than in mammals and that increase of temperature or oxygen pressure, increases the amount oxidized. Taschino¹¹ has shown definitely that oxidation takes place in the nervous tissues. These facts together with the results of our experiments offer a satisfactory explanation of morphine tetanus.¹²

The theory of Melzer and Joseph¹³ that the blood contains a hypothetical antitoxic substance for morphine has been shown unnecessary by Abel¹⁴ who has also shown that tetanus is developed only after the central nervous system contains a certain minimal amount of the tetanic substance. We accept Abel's explanation with the modification that not only is a certain concentration of the drug in the nerve centers necessary, but in the case of morphine, after a certain oxidation of it has taken place. This oxidation occurs in mammals so rapidly that relatively a

⁷ Frenkel: *Ibid.*

⁸ Cloetta: *Archiv f. exp. Path. u. Pharmacologie*, 1903, I, 453.

⁹ Faust: *Archiv f. exp. Path. u. Pharmacologie*, 1900, xliv, 217.

¹⁰ Frenkel: *loc. cit.*

¹¹ Taschiro: *American Journal of Physiology*, 1913, xxxii, 107.

¹² Asphyxiation, as suggested by Abel (*loc. cit.*), by increasing the sensitivity of the cord, may be a factor in the reaction. This however will fit any of the theories mentioned.

¹³ Meltzer and Joseph: *loc. cit.* Also Githens and Meltzer, *Zentralblatte für Physiologie*, xxvi, no. 3, p. i.

¹⁴ Abel: *Jour. of Pharm. and Exper. Ther.*, 1911, iii, 581.

very large amount of the morphine must be given in order to elicit convulsions and special means used to protect the respiratory center from the direct action of the morphine itself.¹⁵ If however the morphine be injected directly into the cerebrospinal fluid, the oxidized product acts at the point of its formation and so tetanus is readily produced. This explanation will also hold good for the cardiectomized frogs in which Meltzer and Joseph found that less morphine was necessary to produce convulsions than in normal animals.¹³

Table 2 includes results with morphine alone, morphine more or less oxidized and also unchanged morphine to which salt of the same kind and amount as that formed in the oxidation, had been added. We do not in any case argue that the oxidation in the body and oxidation by nitric acid *in vitro* are the same but the results of their action in animals suggest similarity.

Solutions 1, 2, 3 and 4 contain the same concentration of morphine sulphate but increasingly oxidized from 1 to 4. Tests 10, 11, 12, 13 and 14 show clearly that morphine may be oxidized first to a more active and later to a less active form. The sedative action varies in the reverse order. Test 15 shows that the salt formed in the process of oxidation hastens the onset of the spasm but the acceleration is relatively insignificant.

In order to get nearer to the mechanism of the general convulsions, morphine and related bodies were injected into the spinal canal of mammals and chiefly in the region of the fourth ventricle. Table 3 summarizes the work in this line. Morphine in doses up to 3 mgms. produces more or less depression but no convulsion. Larger doses after a lapse of time produce tetanus. These spasms may be explained in the same way as in frogs, i.e., not to morphine *per se* but to an oxidation product of it. Tests 10 to 17 support this contention.

¹⁵ Gordon: Philadelphia Medical Journal, March 21, 1903 and Keen, *ibid.*, March 28, 1903, record cases in which pain, apparently, may so neutralize the action of morphine on the higher centers that the oxidized products may reach sufficient concentration to produce tetanic symptoms in men. Quoted from a note by Dr. Alfred Gordon, Journal American Medical Association, 1915, lxiv, 1367.

It is also shown that compounds related to morphine such as codeine, apocodeine and morphine when injected into the fourth ventricle of does do not produce convulsions. The artificially oxidized products of these bodies however do bring about tetanus very quickly. This agrees with the results of Bouma¹⁶ and of Babel¹⁷ who has shown that codeine is not oxidised in the body while morphine is oxidized. This inability of the body to oxidize codeine and the consequent failure of it to produce tetanus agrees with our explanation of morphine tetanus.¹⁸

From the work recorded the following conclusions may be drawn:

1. Morphine when injected intraspinally into mammals (dogs and cats) in adequate doses will produce tetanus after a latent period. Codeine which is apparently not oxidized in the body will not produce tetanus.

2. Morphine when oxidized to a certain degree and injected into the spinal canal of dogs or cats, or into the lymph of frogs, will produce tetanus more quickly than unchanged morphine. Artificially oxidized codeine will also produce tetanus when injected in this way.

3. Oxidized morphine when administered otherwise than directly into the cerebro-spinal fluid will not produce convulsions in mammals (dogs).

4. The strychnine like action of morphine is apparently due to an oxidation product which is first produced and then destroyed in the body. Differences in the rate of oxidation will explain the varied reaction in different animals.

¹⁶ Bouma: *Archiv f. exp. Path. u. Pharmacologie*, 1903, I, 353.

¹⁷ Babel: *Archiv f. exp. Path. und Pharmacologie*, 1905, lii, 262.

¹⁸ Codeine produces tetanus in frogs more quickly than morphine. In dogs however 2 cc. of 1 per cent codeine sulphate in the fourth ventricle will not produce tetanus in a 10 kilo animal. Oxidized codeine will elicit tetanus quickly. We are unable to explain the variations in the action of codeine. Its pharmacology is unsatisfactory and many of the reported actions of it may be due to adherent impurities. The action of codeine requires investigation.

THE SYNERGISM OF MORPHIN AND THE SCOPOLAMINS

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In 1900 Schneiderlin observed that the narcotic effect of scopolamin was increased when administered in combination with morphin. He observed that the narcosis which developed subsequent to the administration of a combination of morphin and scopolamin was so deep that he was led to its use to induce anesthesia in operative cases. Later Korff¹ made a further clinical study of this combination, and while he modified the original dosages of Schneiderlin, he agreed with the former observer, both as to the synergism of morphin and scopolamin with regard to their narcotic effects, as well as to their antagonistic properties in general.

Steinbüchel² subsequently extended the use of the scopolamin-morphin combination to effect partial narcosis during labor. This led Gauss³ to a very extensive clinical study of these drugs in Krönig's institute at Freiburg, especially with reference to their dosage and administration, which resulted in the creation of the so-called "Twilight Sleep" in obstetrics.

Numerous clinicians have since used the scopolamin-morphin combination in obstetrics, as a preliminary hypnotic to general anesthesia in operative cases, and even as a general anesthetic in place of ether or chloroform. The clinical reports vary. Some observers claim favorable results, others are more reserved, while many have proclaimed this combination as positively dan-

¹ Korff: Münch Med. Woch., 1903, v. 50.

² Steinbüchel: Beitr. z. Geburtsh. und Gynäk., Chrobach's Festschr., 1903.

³ Gauss: Arch. f. Gynäk, 1906, v. 78.

gerous. Thus Reichel⁴ reports two fatalities due to the scopolamin-morphin narcosis, and he cites Bruestein having reported three fatalities, Krauss two, Zahradnicky a case of degeneration of the heart, and several other observers having reported cases of oligopnea resulting from its use.

Kochman⁵ states it is a dangerous anesthetic, giving a frequency of fatalities of 1:100 as compared with 1:5000 and 1:2000 for ether and chloroform respectively.

Brunner⁶ regards the scopolamin-morphin combination dangerous. He reports two cases of respiratory failure caused by 0.04 g. pantopon and 0.0004 g. scopolamin, and by 0.02 g. pantopon and 0.0006 g. scopolamin respectively. He concludes that scopolamin and morphin are, contrary to the teachings of Schneiderlin and Korff, not antagonistic in their effects on the respiratory center, but probably synergistic.

Bosse,⁷ Zweifel,⁸ Bass,⁹ Hocheisen¹⁰ and others who have used the scopolamin-morphin combination in obstetrics report from 10 to 20 per cent of non-fatal asphyxias and a variable percentage of fatalities in the new-born. That this may be the direct effect of the scopolamin-morphin narcosis is made highly probable by the fact that Holzbach¹¹ demonstrated the presence of scopolamin in the urine of the new-born in "Dämmer Schlaf" cases.

Numerous as the clinical observations have been, very little seems to have been done with scopolamin-morphin combinations in the laboratory. Bürgi¹² showed that scopolamin and morphin are synergistic in their narcotic effects on rabbits. Madelung¹³ also demonstrated on rabbits that scopolamin augments the nar-

⁴ Reichel: Münch. Med. Woch., 1913, v. 60.

⁵ Kochmann: Ibid., 1905, v. 52.

⁶ Brunner: Ibid., 1912, v. 59.

⁷ Bosse: Monatsch. f. Geburtsh. und Gynäk., v. 30, p. 316.

⁸ Zweifel: Ibid., 1913, v. 36, p. 258.

⁹ Bass: Münch. Med. Woch., 1907.

¹⁰ Hocheisen: Ibid., 1907, v. 54.

¹¹ Holzbach: Ibid., 1907, v. 54, p. 1228.

¹² Bürgi: Deut. Med. Woch., 1910, v. 36, p. 20.

¹³ Madelung: Arch. f. Exp. Path. und Phar., 1910, v. 62, p. 409

cotic properties of morphin, and that a moderate dose of the former will considerably reduce the minimal effective dose of the latter.

The aim of the present investigation has been to determine whether the synergism of scopolamin and morphin is limited to their narcotic effects only, or whether it is more general in its scope. It was also desirable to find out whether there is any difference in the optically different scopolamins when combined with morphin.

Two scopolamins were therefore used in the course of this work; inactive scopolamin hydrobromide or atropine, and levorotary scopolamin hydrobromide. The specimen of i-scopolamin hydrobromide used had been prepared by Cushny¹⁴ from hyoscyne hydrochloride of Schuchardt. The l-scopolamin hydrobromide, which is generally used clinically, was obtained from Merck. An optical examination of a 4 per cent aqueous solution of this specimen gave $(\alpha)_D^{21} = -19.5$.¹⁵ Fresh solutions of the scopolamins were made each time to preclude the possibility of deterioration.

The experiments were first made on intact frogs, then the effect of the combined drugs on the heart was studied by perfusion of the isolated frog's heart, finally it was thought advisable to extend the observations to warm blooded animals, for which purpose white mice were chosen.

INTACT FROGS

The frogs used in these experiments were *Rana pipiens*. They were chosen of fairly uniform size (the average weight being about 30 grams), weighed and the drugs injected into the anterior, or anterior and lateral lymph sacs, in case more than one drug were injected, from a 1 cc. pipette graduated into 0.01 cc. and connected with an air pressure bottle. The solutions used were of such concentration that at no time did the total volume injected ex-

¹⁴ Cushny and Peebles: Jour. Physiol., v. 32, p. 501.

¹⁵ For the optical examination of the scopolamins I am indebted to Mr. Clifford C. Glover.

ceed 1 cc. The quantities of drugs injected are expressed in terms of mgm. per gram of body weight.

In a preliminary study of the individual drugs on the frog an attempt was made to determine the minimal effective dosages that would produce characteristic well defined symptoms whenever uniformity of results would permit. It was found that hyperexcitability, tonic convulsions, and death were each produced with remarkably uniform dosages of morphin sulphate.¹⁶ Thus table I giving the results with morphin shows that hyper-

TABLE I
Morphin sulphate on Rana pipiens

NUMBER OF EXPERIMENTS	MGM. PER GRAM	ONSET OF HYPEREXCITABILITY AVERAGE IN HOURS	ONSET OF CONVULSIONS AVERAGE IN HOURS	DURATION OF HYPEREXCITABILITY OR CONVULSIONS AVERAGE IN HOURS	RESULTS
3	0.25				No effect
3	0.3				One showed very slight hyper excitability
5	0.4	3		20	One showed no effect
5	0.5	4		47	
5	0.6	2½	8	57	2 out of the 5 had convulsions.
5	0.7	3	8	72	3 had convulsions. All survived
4	0.75	2	7	70	All died
3	0.8	1½	6	50	All died

excitability is produced by 0.4 mgm. per gram, convulsions by 0.6 mgm. per gram, while the minimal lethal dose is 0.75 mgm. per gram.

The characteristic symptom of peripheral motor paralysis produced in the frog by members of the atropin group was also found to result with constant uniformity from definite amounts of the scopolamins. Thus table II summarizing the results of the experiments made with i-scopolamin hydrobromide shows that the minimal paralytic dose is 0.5 mgm. per gram and that the minimal fatal dose is 2.3 mgm. per gram. The results ob-

¹⁶ Spring frogs are more susceptible to morphin and do not yield so uniform results as winter frogs.

tained with l-scopolamin hydrobromide are essentially the same, although this drug is much less toxic to the frog. The minimal effective paralytic dose of this drug lies between 0.9 and 1.0 mgm. per gram while the minimal lethal dose is close to 4.0 mgm. per gram.

In table III the results obtained in a study of the combined drugs in sublethal doses are presented; subdivision A showing the effects of i-scopolamin and morphin combinations, B giving

TABLE II
i-Scopolamin hydrobromide on Rana pipiens

NUMBER OF EXPERIMENTS	MG. PER GRAM.	PARALYSIS		RESULTS
		Onset min.	Duration hours	
4	0.3			No effect
8	0.4	60	3	5 of the 8 showed no effects
3	0.45	60	1	2 of the 3 showed no effects
4	0.5	60	3	All had paralysis
2	0.6	60	5	All had paralysis
2	0.9	30	4	All had paralysis
2	0.95	30	6	All had paralysis
2	1.0	30	5	One died
2	1.5	20	10	Recovered
2	2.2	10	24	Recovered
1	2.3	8		Died
1	2.4	10		Died
1	2.5			Died

those of l-scopolamin and morphin combinations. This table clearly demonstrates that even small doses of the scopolamins reduce the effective dose of morphin, as is shown in experiments 2 and 4 of III A and in series 1 of III B, where 50, 45 and even 40 per cent of the effective dose of the scopolamins were sufficient to produce hyperexcitability in the frog with 0.2 mgm. per gram of morphin which is only 50 per cent of the effective dose of the latter. It also appears that morphin has a similar effect on the scopolamins, although relatively much larger doses of morphin are required to induce susceptibility in the frog to the scopolamins. Thus experiments 11, 13 and 18 of A and series 3 of B show that only with as large quantities of morphin as 125 per

cent and 150 per cent of the effective dose could paralysis be induced with 75 per cent, 60 per cent and even 40 per cent of the effective dose of the scopolamins.

TABLE III

Scopolamins and morphin in combined sublethal doses on Rana pipiens
A l-scopolamin hydrobromide

EXPER. • NO.	MORPHIN SULPH.		SCOPOLAMIN HYDROBROMIDE		HYPER- EXCITABILITY IN HOURS		PARALYSIS IN HOURS		RESULTS
	Mgm. per gram	Percent of ef- fective dose	Mgm. per gram	Percent of ef- fective dose	Onset	Dura- tion	Onset	Dura- tion	
1	0.1	25	0.5	100			2	2	Recovered
2	0.2	50	0.25	50	5	*			Recovered
3	0.2	50	0.5	100	7	48	2	1½	Recovered
4	0.2	50	0.2	40	7	24			Recovered
5	0.2	50	0.1	20					Recovered
6	0.3	75	0.4	80	4	80			Recovered
			0.5	100			½	*	
7	0.4	100	0.5	100	4	24	1	2	Recovered
			0.3	60					
10	0.5	125	0.3	60	5	24			Died
11	0.6	150	0.3	60	4	*	½	2	Died
12	0.7	175	0.1	20	4	*			Died
13	0.5	125	0.2	40	5	120	1½	10	Recovered
14	0.5	125	0.1	20	5	168			Recovered
18	0.6	150	0.3	60	2	53	½	1½	Died
19	0.3	75	1.0	200	6	90	½	1½	Recovered

B l-scopolamin hydrobromide

SERIES	NUM- BER OF EXPERI- MENTS								
1	4	0.2	50	0.45	45	*	48		One showed no effect.
2	3	0.3	75	0.45	45	*	48		All recovered
3	3	0.5	125	0.75	75	*	72	1	All had paral- ysis; 2 died, one recover- ed

*Not observed.

A somewhat similar condition appears from the experiments grouped in table IV. Thus experiments 13 and 14 of IV A show that typical morphin convulsions could be induced with 0.5 mgm. per gram or 83 per cent of the effective convulsive dose of morphin if even very small ineffective doses (0.2 and 0.1 mgm. per

gram) of *i*-scopolamin were injected simultaneously. When larger, effective doses of the scopolamins were injected, the effective convulsive dose of morphin could even further be reduced as is

TABLE IV
Scopolamins and morphin in combined lethal doses on Rana pipiens
A i-scopolamin hydrobromide

	EXPERIMENT NO.	MORPHIN SULPH.		SCOPOLAMIN HYDROBROMIDE		TOTAL PER CENT	CONVULSIONS IN HOURS		RESULTS
		Mgm. per gram	Per cent of fatal dose	Mgm. per gram	Per cent of fatal dose		Onset	Duration	
	8	0.5	66	0.5	22				
				0.5	22	110	23	55	Died
	9	0.6	80	0.5	22	102		30	Died
	10	0.5	66	0.3	13	79			Died
	11	0.6	80	0.3	13	93	7	7	Died
	12	0.7	93	0.1	4	97	24	8	Died
	13	0.5	66	0.2	8	74	23	29	Recovered
	14	0.5	66	0.1	4	70	22	49	Recovered
	15	0.4	53	0.9	39	92	22	54	Recovered
	16	0.4	53	1.0	44	97	22	30	Recovered
	17	0.5	66	0.05	2	68	29	2½	Died
	18	0.6	80	0.3	13	93	23	33	Died
	19	0.3	40	1.0	43	83			Recovered
	20	0.5	66	1.5	65	131		24	Died
	21	0.5	66	2.0	87	153			Died
	22	0.4	53	2.0	87	140			Died

B l-scopolamin hydrobromide

SERIES	NUMBER OF EXPERIMENTS								
1	4	0.5	70	1.0	25	95	24	48	3 died, all had convulsions
2	4	0.4	57	1.5	37	94	24	24	2 died, 3 had convulsions
3	3	0.38	50	2.0	50	100	72	10	All died, one had convulsions
4	3	0.56	75	1.0	25	100	9	48	1 died, 2 had convulsions
5	2	0.45	60	1.6	40	100	10	24	All died, all had convulsions
6	1	0.19	25	3.0	75	100			Died
7	3	0.5	70	0.75	20	90	14	72	2 died, 1 had convulsions

seen in experiments 15 and 16 of IV A and in series 2 and 3 of IV B where the convulsive dose of morphin was reduced to 0.4 mgm. per gram or 67 per cent of the effective dose. No convulsions are

recorded in experiments 21 and 22 probably because the frogs did not live long enough for the paralytic effect of the i-scopolamin to wear off and for the convulsions to set in.

That the scopolamins render the frog susceptible to subeffective doses of morphin while it takes comparatively large quantities of the latter to induce at most only slight susceptibility to the scopolamins appears to be a contradiction at first sight. This, what would seem to be a one-sided synergism, is readily explained by the fact that the manifestations produced by morphin, viz., hyperexcitability and convulsions are of central origin while the paralysis produced by the scopolamins in the frog is peripheral. The scopolamins however also undoubtedly exert some central action in *Rana pipiens*, as is shown by the cessation of respiration and frequently by a central paralysis preceding the peripheral paralysis especially when large doses of the scopolamins are injected. It is to this extent that they are synergistic with morphin. Morphin on the other hand, is not synergistic with the scopolamins in producing paralysis, for, as it has been stated above, the paralysis is of peripheral origin mainly. It becomes clear then that the most one could obtain with large doses of morphin is only a slight susceptibility to the paralytic effects of the scopolamins, but not a true synergism.

That the scopolamins are synergistic with morphin in combined lethal doses is very clearly shown in table IV A and B, the combined toxic dose being close to 100 per cent. It also appears that the two scopolamins bear essentially the same relation to morphin.

PERFUSION EXPERIMENTS ON THE ISOLATED FROG'S HEART

It next seemed advisable to determine what the combined effect of the scopolamins and morphin is on the heart. With this aim in view perfusion experiments on the frog's heart were undertaken. Larger sized frogs, weighing about 75 grams, of as uniform size as possible, were used in these experiments. The perfusion fluids (Ringer's solution, and a solution of the drugs studied in Ringer's fluid) were supplied from a double reservoir

fitted with a 3-way stopcock and connected with a cannula introduced into the ascending vena cava. The fluid returned from the heart by way of a cannula introduced into the bulbous aorta.



FIG. 1. PERFUSION OF FROG'S HEART

Shows minimal effect produced by 1:500 solution of morphin sulphate. Upstroke = systole.

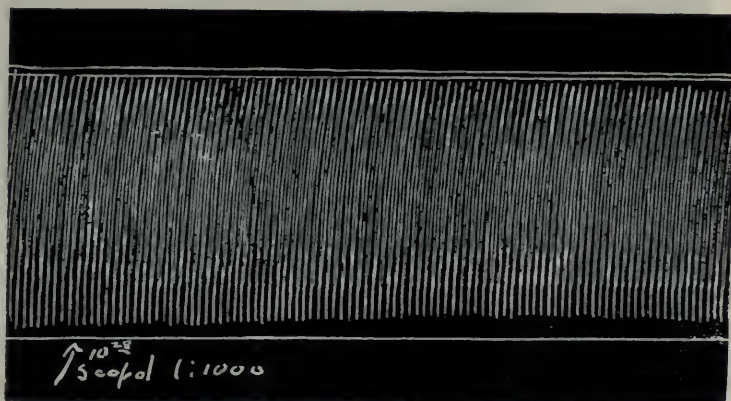


FIG. 2. PERFUSION OF FROG'S HEART

Minimal effect of 1:1000 solution of i-scopolamin hydrobromide. Upstroke = systole.

The apex of the heart was connected with the recording lever by means of a clasp protected with a silk thread cushion. Experiments were made to determine the minimal effective dilutions of the scopolamins and of morphin respectively. Then various

combinations of these drugs were tried. A few of the tracings obtained are here reproduced to show the results. Tracing I shows that 1:500 solution of morphin sulphate is the lowest dilution that will have a definite effect on the frog's heart. Tracings taken with dilutions of 1:1000 show no effects. The minimal effective dilution of i-scopolamin hydrobromide, as is shown in tracing II, is 1:1000 while that of l-scopolamin hydrobromide is 1:1500 as appears from tracing III. Tracing IV representing a combination of morphin 1:1500 and i-scopolamin 1:2000, neither of which is effective individually, shows a very definite effect on

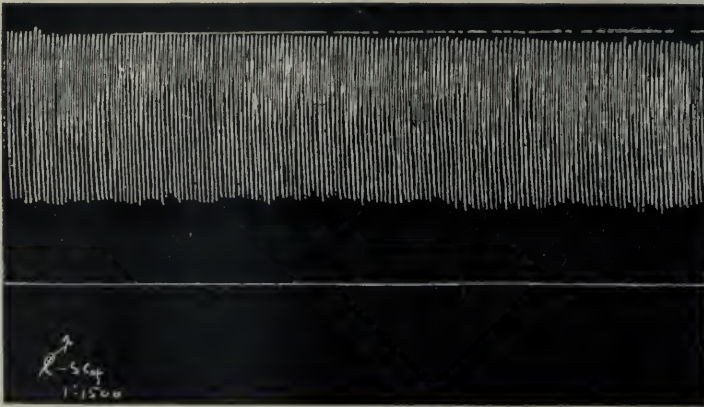


FIG. 3. PERFUSION OF FROG'S HEART

Shows minimal effect of 1:1500 solution of l-scopolamin hydrobromide. Up-stroke = systole.

the heart muscle as is evidenced by the markedly reduced amplitude of the heart beat. Similarly dilutions of l-scopolamin 1:3000 and morphin 1:1000, not effective individually, are effective in combination as is shown in tracing V. It seems then only reasonable to conclude that morphin is synergistic with the scopolamins in its action on the frog's heart.

This is not at all surprising in view of the results obtained with the intact frog. Since respiratory movements are not a very important factor in the frog's economy, the synergistic action of morphin with the scopolamins as is shown in table IV could not

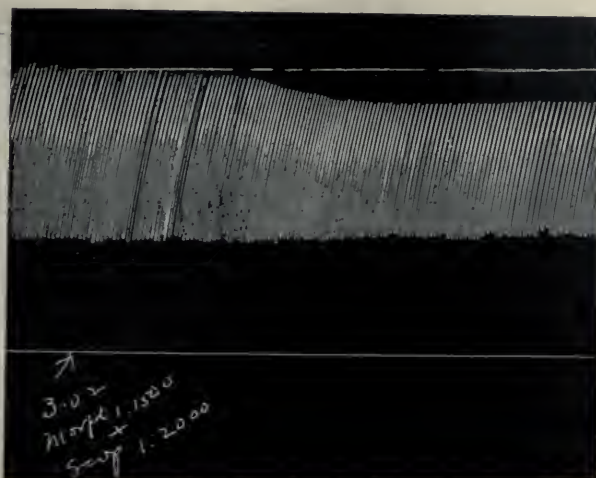


FIG. 4. PERFUSION OF FROG'S HEART

Shows the effect of a combination of morphin sulphate 1:1500 and i-scopolamin hydro-bromide 1:2000. Upstroke = systole.

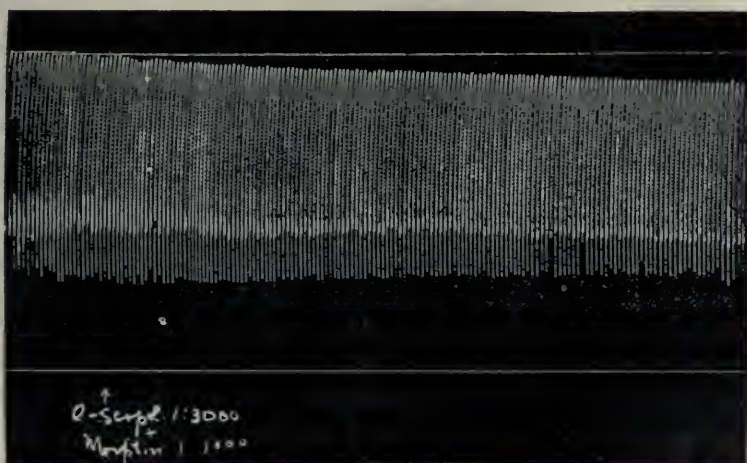


FIG. 5. PERFUSION OF FROG'S HEART

Effect of a combination of morphin sulphate 1:1000 and l-scopolamin hydro-bromide 1:3000. Upstroke = systole.

wholly be explained by their action on the respiratory center. That the heart therefore might also be affected synergistically by the combination of these drugs was surmised. The results of the perfusion experiments certainly bear out such supposition.

MICE

In order to determine definitely whether the scopolamins and morphin have any effect upon each other in their action on the respiratory center it was necessary to extend the experiments to warm blooded animals. White mice of one litter and of fairly uniform weight were used in each of the two groups of experiments. They were kept on a constant diet throughout each group of experiments, for as Reid Hunt¹⁷ has shown the toxic dose of a drug in the mouse may vary with its diet. The mice were weighed and the drugs, calculated in mgm. per gram of body weight, were injected subcutaneously, the total volume injected never exceeding 1 cc. Preceding each group of experiments (A and B of table V) the minimal lethal doses of morphin sulphate and of one of the scopolamins were determined by a number of experiments. Thus preliminary to the experiments of group A (table V) the minimal lethal dose for morphin sulphate was found to be 0.2 mgm. per gram, while that for i-scopolamin hydrobromide was 0.7 mgm. per gram. Similarly a series of experiments were performed, preliminary to those of group B (table V), on white mice of a different-litter with the result that the minimal lethal dose for morphin sulphate was 0.46 mgm. per gram while that of l-scopolamin was 1.3 mgm. per gram. Death was caused in all cases by respiratory failure, for the heart could be felt beating some time after respiration had ceased. It may be stated here parenthetically that at the time when the experiments of group B with l-scopolamin were carried out, control experiments were made with the i-scopolamin hydrobromide on the mice of this litter and its minimal lethal dose was exactly the same, as that of the l-scopolamin, viz. 1.3 mgm. per gram, so that there is no quantitative difference between the two scopo-

¹⁷ Reid Hunt: Hygienic Lab. Bull., No. 69.

lamins in their toxicity nor could there be made out any qualitative difference in their action on the central nervous system of the mouse.

In table V A the results obtained from various combinations of i-scopolamin hydrobromide and morphin sulphate are summarized; the results of combined doses of l-scopolamin and mor-

TABLE V
Scopolamins and morphin in combined doses on mice
A i-scopolamin hydrobromide

SERIES NO.	NUM-BER OF EXPERI-MENTS	MORPHIN SULPH.		SCOPOLAMIN HYDROBROMIDE		TOTAL PER CENT	RESULTS
		Mgm. per gram.	Per cent of fatal dose	Mgm. per gram.	Per cent of fatal dose		
1	4	0.15	75	0.18	25	100	One died, three recovered
2	4	0.12	60	0.28	40	100	Two died, two recovered
3	10	0.1	50	0.35	50	100	Five died, five recovered
4	11	0.08	40	0.42	60	100	Four died, seven recovered
5	3	0.06	30	0.49	70	100	All died
6	4	0.05	25	0.53	75	100	All died
7	2	0.1	50	0.3	43	93	One died, one recovered
	1	0.05	25	0.4	57	82	Recovered
8	1	0.05	25	0.5	71	96	Recovered
	1	0.075	38	0.5	71	109	Died
	1	0.1	50	0.5	71	121	Died
	1	0.15	75	0.3	43	118	Died
	1	0.1	50	0.4	57	107	Recovered

B l-scopolamin hydrobromide

1	8	0.23	50	0.65	50	100	All died
2	4	0.20	45	0.65	50	95	All died
3	5	0.20	45	0.585	45	90	All recovered
4	6	0.09	20	0.91	70	90	3 died, 3 recovered

phin are shown in table V B. It is interesting to note that the synergism between i-scopolamin and morphin is more complete as the relative content of the i-scopolamin dose is increased. Thus in series 1 of table V A where 75 per cent of the fatal dose of morphin was combined with only 25 per cent of the fatal dose of i-scopolamin, only one out of the four died. In series 2 and 3

where the scopolamin content of the combined fatal dose was increased to 40 per cent and 50 per cent of its fatal dose, 50 per cent of the mice in each series died. Finally those of series 5 and 6 that received 70 per cent and 75 per cent of the fatal dose of i-scopolamin with 30 per cent and 25 per cent respectively of morphin all died. Series 4 presents a slight deviation which cannot be explained satisfactorily. A glance at series 7 and 8 of the same table shows that combined sublethal doses are generally not fatal, while combined doses exceeding 100 per cent are generally fatal.

An analysis of table V B will show that l-scopolamin when combined with morphin is more toxic than the i-scopolamin. Thus in series 1 and 2 of table V B all the mice having received 100 per cent and even 95 per cent of the combined fatal dose died. Series 3 and 4 of the same table further demonstrate that the toxicity of the combined drugs is increased with the increase of the scopolamin content of the combined dose. Thus in series 3 where 45 per cent of each were given all recovered, while in series 4 where the same combined dose of 90 per cent was given but the scopolamin content was raised to 70 per cent, half of the mice died.

In view of the above findings it would seem that scopolamin is the more dangerous of the two drugs, it becoming especially toxic as its relative content is increased. While it may be wise to keep the morphin content of this combination at a safe level, as it is done in the clinical use of scopolamin-morphin combinations, the repeated injections of scopolamin almost as often as necessary, as it has been carried out recently in many clinics, would not seem to be without danger.

SUMMARY

The scopolamins are synergistic with morphin in the frog, both in combined sublethal doses as well as in combined lethal doses.

There is a synergistic action between the scopolamins and morphin on the frog's heart.

The scopolamins are synergistic with morphin in the mouse, probably through their combined action on the respiratory center.

The toxicity of the scopolamin-morphin combination in the mouse is increased with the relative increase of the scopolamin content of the combined dose.

l-Scopolamin is more toxic to the mouse in combination with morphin than i-scopolamin. In other respects the two scopolamins are essentially alike in their relation to morphin.

ON THE ACTION OF COCAINE

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The dilatation of the pupil and other effects of cocaine when applied locally to the eye appeared so similar to the changes following stimulation of the cervical sympathetic trunk, that it was early suggested that cocaine stimulates the terminations of the postganglionic sympathetic fibres. The earlier experiments in support of this view are given by Limbourg¹ who also adds some observations of his own. As a general rule he found like his predecessors that when the superior cervical ganglion was extirpated and time was allowed for the degeneration of the fibres, cocaine failed to cause dilatation of the pupil, but in some cases the action persisted or could be reinstated by special measures. Schultz² also found that ordinary quantities of cocaine failed to dilate the pupil after degeneration, but larger doses still had this effect, from their paralysing the terminations of the short ciliary fibres, as he assumed. The examination of the action of adrenaline on the iris, however, shows that the degeneration of the postganglionic fibres does not necessarily involve the terminations, and it can no longer be argued that because the cocaine dilatation of the pupil does not occur after degeneration of these fibres in many cases, these terminations must be the site of its action. Many of the observations on which the prevailing view of the cocaine mydriasis is founded may be explained equally readily by its depressing the sphincter muscle of the iris; Schultz considers this possibility, but rejects it on the ground that the muscle of the frog's stomach was unaffected by even 5 per cent cocaine solution; but this argument is equally valid

¹ Arch. f. exp. Path. u. Pharm., 1892, xxx, p. 115.

² Arch. f. Anat. u. Physiol., 1898, p. 58.

against its action on the sympathetic nerve ends in the iris, for it might be expected to act equally strongly on the gastric nerve ends. Sighicelli³ had previously stated that cocaine applied to the rabbit's eye paralyses the smooth muscle of the iris, which no longer contracted on direct stimulation by an electric current. Froehlich and Loewi⁴ state that very small quantities of cocaine augment the adrenaline action on the blood pressure, iris, and bladder, but not on the salivary secretion, but this does not necessarily entail that they act on the same structures. There does not seem to be much prospect of determining the question by further examination of the action on the iris, in regard to which the observations are fairly agreed, but it seemed desirable to determine more exactly the effects of cocaine on other forms of unstriated muscle and to compare them with those of adrenaline as the typical sympathetic-mimetic reagent. If in other forms the action of cocaine and adrenaline proved similar, there would be a strong ground to believe that they affect the same structures or nearly related structures in the iris. Some previous observations seemed to indicate a certain similarity in action and these will be taken up in regard to each organ.

ACTION ON THE BLOOD VESSELS

Cocaine is frequently said to contract the vessels by direct action on their muscular walls, but in experiments by perfusion through surviving organs most authors have failed to observe any constriction (Kobert,⁵ Mosso⁶); Brodie and Dixon⁷ found some transient constriction from cocaine, but the prevailing effect was dilation of the vessels, as had been previously noted by Mosso under large doses. This dilation of the vessels by cocaine weakens to some extent the subsequent constriction by adrenaline (Brodie and Dixon, Laewen⁸). On the other hand

³ Cited by Albertoni: *Arch. f. d. ges. Physiol.*, 1891, xlviii, p. 307.

⁴ *Arch. f. exp. Path. u. Pharm.*, 1910, lxii, p. 159.

⁵ *Arch. f. exp. Path. u. Pharm.*, 1887, xxiv, p. 100.

⁶ *Ibid.*, 1887, xxiii, p. 153.

⁷ *Journ. of Physiology*, 1904, xxx, p. 498.

⁸ *Arch. f. exp. Path. u. Pharm.*, 1904, li, p. 432.

it is a common clinical observation that cocaine contracts the vessels of the conjunctiva when it is applied locally in about the same concentration as was used by these writers for perfusion.

I have made a number of experiments with the hope of removing this discrepancy between clinical and experimental observation.

I. The mesenteric vessels of a completely pithed frog (*Rana temporaria*) were observed under the microscope, care being taken to protect the mesentery from drying. Cocaine hydrochloride was applied in strengths varying from 0.2 per cent to 0.05 per cent and uniformly was followed by relaxation of the vessels and acceleration of the blood current. Solutions under 0.05 per cent had no apparent effect. Adrenaline solution 1 in 1,000,000 applied locally to the mesentery narrowed the vessels and completely arrested the blood circulation in it, but it could be restored by the subsequent application of 0.2 per cent cocaine.

II. The rate of perfusion in the frog (*Rana temporaria*) was measured by Trendelenburg's⁹ method, and the results obtained were in accord with those obtained by direct observation of the vessels. I have put the results of a number of experiments in table I, from which it is seen that cocaine in concentrations lower than 1 in 10,000 has no effect, while in those stronger than 1 in 5000 the blood vessels are relaxed and in consequence the number of the drops from the vein cannula is increased. In my experiments I never found the number of drops decrease whether under a high or a low pressure (table I).

Anrep¹⁰ states that cocaine injected hypodermically in the frog caused immediate constriction of the vessels of the tongue and the interdigital membrane, but this may arise from central action or from weakness of the heart and cannot be regarded as evidence of direct action on the vessels.

III. Ringer's solution or blood was perfused through the kidney or the hind leg of the cat in a number of experiments and

⁹ Arch. f. exp. Path. u. Pharm., 1910, lxiii, p. 161.

¹⁰ Pflueger's Arch. f. Physiol, 1880, xxi, p. 56.

TABLE I

NUMBER OF DROPS UNDER NORMAL CONDITION			STRENGTH OF COCAINE SOLUTION	NUMBER OF DROPS AFTER THE APPLICATION OF COCAINE			
1st $\frac{1}{2}$ min.	2d $\frac{1}{2}$ min.	3d $\frac{1}{2}$ min.		1st $\frac{1}{2}$ min.	2d $\frac{1}{2}$ min.	3d $\frac{1}{2}$ min.	4th $\frac{1}{2}$ min.
24	24		1 in 100	25	26	26	
18	18		1 in 100	24	27	26	
15.5	15.5	15.5	1 in 100	16	18.5	17.5	17.5
11	11	11	1 in 200	11	13	14	13
11	11	11	1 in 200	12	12	11	11
23	24		1 in 200	26	27	26	26
14	14	14	1 in 600	13.5	16	17	17
28	28	28	1 in 1000	28	32	32	34
30	29	29	1 in 2000	30	32	34	34
21	21	20	1 in 3000	20	21	24	24
23	23		1 in 4000	23	24	24	24
11.5	11.5	11	1 in 5000	11	11	11.5	11.5
17	17	17.5	1 in 10000	17	17	17.5	

cocaine was added to the solution when the rate of perfusion had become constant. The fluid and the preparation were kept at body temperature throughout, and the fluid escaping from the vein was measured in drops or in cubic centimeters before and after the addition of cocaine. The results of a number of experiments on the kidney are given in table II, from which it appears that cocaine in 1 per cent solution dilates the vessels slightly, while in 0.2 per cent the results are doubtful. The perfusion of

TABLE II

Cat's kidney (pressure 1.7 meter H₂O)

NUMBER OF DROPS UNDER NORMAL CONDITION, IN PERIODS OF 5 SEC.			DEGREE OF CONCENTRATION OF THE COCAINE	NUMBER OF DROPS AFTER THE APPLICATION OF THE COCAINE SOLUTION, IN PERIODS OF 5 SEC.			
I	II	III		I	II	III	IV
30	29		1 in 100	30	32	31	30
31	31		1 in 100	32	32	31	30
47	47		1 in 100	48	48	46	47
21	21		1 in 500	21	21	22	
26	26		1 in 500	26	27	27	26
27	27	27	1 in 1000	27	27.5	27	

the cat's hind leg gave similar results. There was no evidence in either series that cocaine lessened the venous outflow at any stage, as Brodie and Dixon seem to have observed occasionally.

These experiments therefore agree with those of most previous investigators that cocaine has no constricting effect on the vessels of either the frog or the cat, but rather tends to relax them when it is present in high concentration.

Yet when a 1 per cent solution of cocaine is applied to the conjunctiva in man, it causes marked ischaemia, as I have convinced myself by personal experience. On dropping the same solution in the cat's eye, I could observe no change in the vessels or in the tint. But in one observation in which the cat's conjunctiva was distinctly congested from some irritation, a few drops of cocaine solution quickly reduced the redness and inflammation. This suggested that cocaine may constrict more distinctly vessels previously dilated.

In several experiments the cat's kidney was first perfused with Ringer's solution containing some sodium nitrite and then cocaine was added to the solution. The perfusion with nitrite dilated the vessels, but cocaine failed to cause any diminution in the fluid from the vein. The same result was obtained in perfusion of the rabbit's ear; cocaine solution up to 0.2 per cent does not constrict the vessels dilated with nitrite, and not infrequently dilates them further. Adrenaline solution on the other hand causes definite vasoconstriction.

These perfusion experiments fail to elucidate the vasoconstriction seen in the human conjunctiva under cocaine, and this failure might suggest that the action was an indirect one through the anaesthesia, were it not that some other anaesthetics have no such constrictor action. The vasoconstriction apparently arises from action on some body which does not survive, or does not react, in vessels perfused with Ringer's solution. It is thus different from the myoneural junction on which adrenaline exerts its action.

ACTION ON THE HEART

Mosso states that the excised and perfused heart of the esculenta is accelerated and strengthened by cocaine and then slowed, which suggests a possible accelerator action in the beginning. In a number of experiments on the heart of *Rana temporaria*, I could detect no phase of acceleration whatever, the earliest effect induced being weakened systole and such irregularities as partial heart block and half rhythm, which were followed by arrest of the heart midway between systole and diastole.



FIG. 1. HEART OF *RANA TEMPORARIA*
Action of cocaine 1 in 10,000

The heart was excised and a circulation with Ringer's fluid containing cocaine instituted through cannulae inserted in the inferior vena cava and aorta (fig. 1). Weaker solutions of cocaine also diminished the strength of the contraction. My results thus agree with those obtained by Anrep in the frog's heart in situ.

In the mammalian heart, Anrep and Mosso observed marked acceleration from moderate quantities of cocaine in the intact animal, especially in the dog. Hedbom¹¹ found no acceleration

¹¹ Skand. Arch. f. Physiol., 1899, ix, p. 66.

nor augmentation in the isolated perfused mammalian heart. Kochman¹² in similar experiments states that the strength of the heart is increased while its rate falls; the electrical excitability of the myocardium seemed increased in some cases. Prus¹³ finds the perfused heart weakened and slowed by the addition of cocaine.

I have performed two experiments on the dog's heart treated by Starling's¹⁴ improved method of "heart-lungs circulation." When cocaine (5 mgm. per kilo body weight) was added to the circulating blood, the vein pressure rose, while the arterial blood pressure did not vary and the output of blood was distinctly lessened. When 10 mgm. per kilo was given, the arterial blood pressure and the output became gradually lower till the death of the animal. The vein pressure, on the contrary, became higher and higher.

These different methods of experimentation all indicate that cocaine fails to accelerate or augment the isolated mammalian heart and it thus differs entirely from adrenaline and other drugs acting on the sympathetic mechanism.

Mosso considered that the rise in blood pressure after cocaine injection arises from its constricting the vessels by direct action on them, though he could find no evidence of this by perfusion experiments. In one experiment on the dog in which he divided the cord between the occiput and atlas however, cocaine prevented the ordinary fall in pressure. In this experiment the blood pressure seems to have been very irregular and it cannot be regarded as decisive. I have done several experiments on cats and dogs decapitated by Sherrington's method and kept alive by artificial respiration. In these the blood pressure was generally 30-40 mm. of mercury, and cocaine injected intravenously in quantities of 1-2 mgm. per kilo had no effect on it. 5-10 mgm. caused a rise of 2-3 mm. in these animals while in a dog with intact central nervous system it raised it about 25 mm. With larger doses than 10 mgm. per kilo, the blood pres-

¹² Arch. internat. de Pharmacodynamique, 1907, xviii, p. 41.

¹³ Zeitschr. f. exp. Path. u. Ther., 1913, xiv, p. 61.

¹⁴ Principles of Human Physiol., 2d edition, p. 911.

sure fell immediately, and in cats even 5 mgm. per kilo was often enough to induce this. The very slight rise in pressure seen under 5–10 mgm. of cocaine in the decapitated dog appears to arise from action on the spinal cord or cardio-inhibitory apparatus, for in atropinised dogs in which the spinal cord was completely destroyed by passing a wire down the canal, this rise

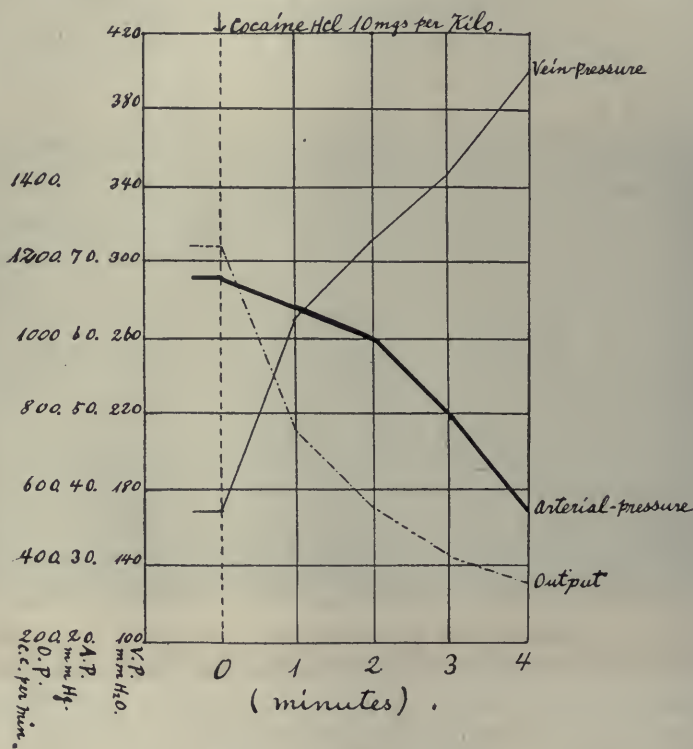


FIG. 2. DOG'S HEART

"Heart-lungs circulation" by Starling's method. Action of cocaine 10 mgm. per kilo.

in the blood pressure was absent. Adrenaline on the other hand gave the typical rise of pressure.

My experiments on the vessels, heart, and blood pressure indicate therefore that cocaine has no definite sympatho-mimetic action on these. The increase in the blood pressure which occurs from its intravenous injection, arises from central action.

ACTION ON THE INTESTINE

Anrep¹⁵ says that cocaine in small doses produces increased movement and in large ones weakens the movement of the exposed intestines in mammals. Bayliss and Starling¹⁶ obtained nearly the same results with the dog's small intestine by the balloon method, and Langley and Magnus¹⁷ confirmed their results in most points in experiments with the balloon method and also with the surviving gut.

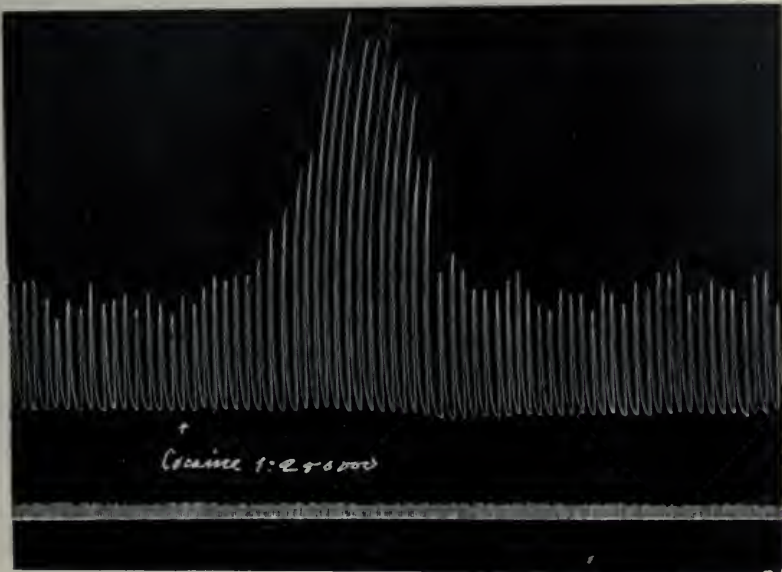


FIG. 3. RABBIT'S SMALL INTESTINE

Suspended in oxygenated Ringer's solution. Cocaine 1 in 250,000

My experiments were performed on pieces of surviving rabbit's intestine suspended in warmed oxygenated Ringer's solution to which cocaine was added in small quantities. The results were uniformly to increase the height of the movement greatly when very dilute cocaine was applied (1 in 250,000, fig. 3).

¹⁵ Pflueger's Arch. f. Physiol., 1880, xxi, p. 67.

¹⁶ Journ. of Physiol., 1899, xxiv, p. 138.

¹⁷ Ibid., 1905, xxxiii, p. 37.

When stronger solutions were used this stage of increased activity was followed by one in which the movements were smaller and the relaxation more complete (1 in 150,000) and when the alkaloid was present in 1 in 10,000 concentration there was no augmentation but an immediate weakening of the movement which soon ended in complete cessation (fig. 4). The effects of cocaine on the intestine are thus entirely different in character from those of adrenaline.

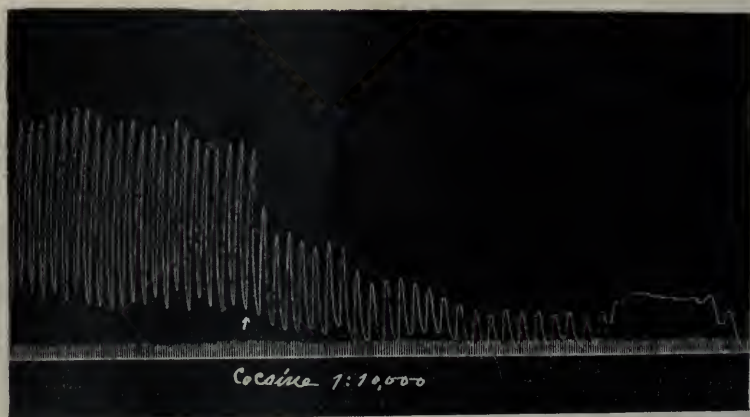


FIG. 4. RABBIT'S SMALL INTESTINE

Suspended in oxygenated Ringer's solution. Cocaine 1 in 10,000

ACTION ON THE STOMACH

The experiments were performed on strips of the isolated stomach of cats by the same method as in the case of the intestine.

The effects of cocaine on the cat's stomach muscle were similar to those on the gut. One in 4000 of cocaine causes a slight contraction of the stomach muscle, 1 in 2000 shows a more apparent contraction, which lasts about 30 seconds or 1 minute, followed by normal movement (fig. 5). When the strength 1 in 1000 of cocaine was applied the contraction of the stomach muscle was more powerful and prolonged, resembling rigor. Adrenaline on the other hand applied in the same way inhibited and relaxed the stomach muscle.



FIG. 5. ISOLATED CAT'S STOMACH MUSCLE
Suspended in oxygenated Ringer's solution. Action of cocaine 1 in 2000



FIG. 6. ISOLATED NON-PREGNANT RABBIT'S UTERUS
Suspended in oxygenated Ringer's solution. Action of cocaine 1 in 10,000



FIG. 7. NON-PREGNANT DOG'S UTERUS
Suspended in oxygenated Ringer's solution. Cocaine 1 in 5000

ACTION ON THE UTERUS

My experiments were made on the isolated uterus of cats, dogs, and rabbits, in the non-pregnant state and also during and after pregnancy. The method employed was similar to that used for the isolated small intestine, the uterus being kept in oxygenated Ringer's solution at 37°C.

As a general rule, the movements recorded were those of the longitudinal fibres, but in some pregnant animals the movement of the circular fibres were recorded. The results of my experiments are in the following:

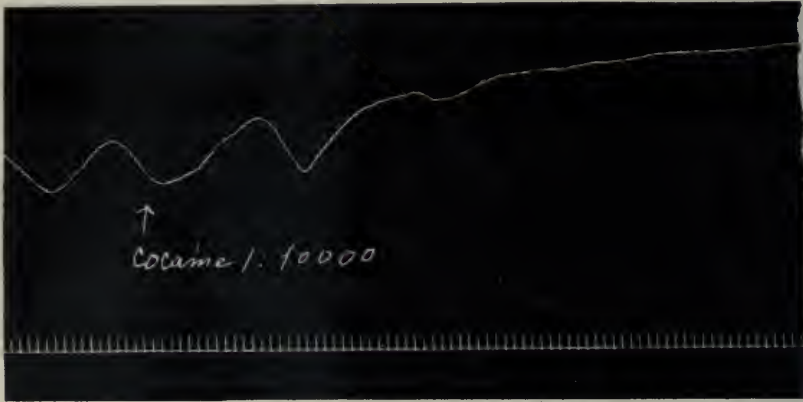


FIG. 8. NON-PREGNANT CAT'S UTERUS

Suspended in oxygenated Ringer's solution. Cocaine 1 in 10,000

I. Non-pregnant rabbit's uterus. One in 10,000 of cocaine hydrochloride causes distinct constriction and augmented movements (fig. 6); when 1 in 1000 of cocaine was applied the constriction is transient and is followed by relaxation and arrest of the movements. One in 1,000,000 of adrenaline augments both the constriction and the movements.

II. Non-pregnant dog's uterus. In the main the uterus was first contracted and then relaxed with cocaine of the strength 1 in 5000 (fig. 7). Adrenaline on the other hand causes a remarkable constriction of the uterus. In a few cases, it was

observed, however, that under cocaine relaxation took place without previous stimulation, and that after adrenaline it became more marked.

III. Non-pregnant cat's uterus. Cocaine (1 in 10,000) augments the constriction in the non-pregnant cat's uterus, sometimes leading to a continued tetanus (fig. 8). Adrenaline in the strength of 1 in 1,000,000 given subsequently to cocaine produces marked relaxation.

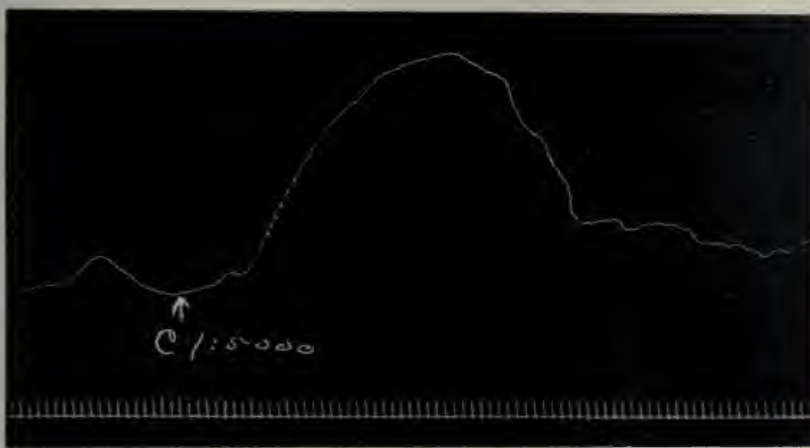


FIG. 9. CAT'S UTERUS DURING PREGNANCY

Suspended in oxygenated Ringer's solution. Cocaine 1 in 5000

IV. Cat's uterus during pregnancy. Both cocaine and adrenaline give rise to contractions of the uterus in the strength of 1 in 5000 of cocaine (fig. 9) and 1 in 1,000,000 of adrenaline.

V. Cat's uterus after pregnancy. Cocaine acts in the same way as in pregnancy, while after adrenaline relaxation takes place after a transitory contraction,

ACTION ON THE URINARY BLADDER

The experiments were performed on cats, and the same method was employed as with the uterus.

The effect of cocaine on the bladder musculature was as follows: a weaker concentration of cocaine than 1 in 3000 does not

affect the bladder, 1 in 2500 of cocaine aguments both its movements and tone, while adrenaline produces inhibition and relaxation (fig. 10).

Thus cocaine departs in many respects from the typical sympathetic-mimetic action of adrenaline, and its action on many organs may be more easily explained by assuming that it affects the muscle directly rather than the nervous mechanism. In the vessels and the heart, the action seems to be purely depression, no evidence of constriction of the former or of increased activity of the heart being presented in my experiments. It is possible, however, that in other conditions some transient



FIG. 10. CAT'S BLADDER

Suspended in oxygenated Ringer's solution. Action of cocaine 1 in 2000

augmentor action may occur, and some of the references in the literature seem to suggest this. In the intestine, stomach, uterus and bladder, the first effect of cocaine in dilute solutions is augmentor, while in more concentrated solution this augmentor action is followed by depression and paralysis. This augmentor action is seen in the stomach, intestine and bladder in which sympathetic stimulation and adrenaline cause inhibition and also in the cat's uterus whether pregnant or non-pregnant; the action of adrenaline in the cat's uterus is to relax the non-pregnant and to contract the pregnant one, so that here again cocaine seems to affect the muscle rather than the nervous apparatus.

These observations that cocaine in other parts of the body acts on muscle rather than on nerve ends suggest a doubt, whether in the iris the dilatation arises from nervous stimulation. It is possible that it stimulates the cervical sympathetic though in other parts of the body it acts on muscle directly, but this does not seem probable without stronger evidence than has been adduced hitherto. It has already been stated that the vessels of the rabbit's ear which like the dilator of the iris are supplied by the cervical sympathetic are not contracted by cocaine. Some experiments were performed to find if it increased the secretion of the submaxillary gland by sympathetic stimulation.

A cat, weight 4350 grams, was anaesthetised with ether, a cannula was inserted in the external jugular and another in the submaxillary duct.

At first I injected 1 mgm. of pilocarpine intravenously in order to test whether the cannula was rightly inserted or not. Atropine 0.1 mgm. stopped the secretion, and then cocaine 2 mgm. per kilo was given intravenously but did not produce any secretion of saliva; but when adrenaline was injected intravenously it produced at the first stage of its action a moderate quantity of a saliva which was not clear but resembled chyle. This flow, however, very quickly decreased.

TIME	THE FLOW OF SALIVA IN CC.	REMARKS
10 h. 30 m.	Pilocarpine 1.0 mgm.	Intravenously
10 h. 30 m.-10 h. 35 m.	3.0	Clear
10 h. 35 m.-10 h. 40 m.	2.8	Clear
10 h. 40 m.	Atropine 0.1 mgm.	Intravenously
10 h. 40 m.-10 h. 45 m.	0	
10 h. 45 m.-10 h. 50 m.	0	
10 h. 50 m.	Cocaine 3.0 mgm. per kilo	Intravenously
10 h. 50 m.-10 h. 55 m.	0	
10 h. 55 m.-11 h.	0	
11 h.	Adrenaline 0.3 mgm. per kilo	Intravenously
11 h.-11 h. 5 m.	0.7	
11 h. 5 m.-11 h. 10 m.	2 drops	
11 h. 10 m.	Cocaine 3 mgm. per kilo	Intravenously
11 h. 10 m.-11 h. 15 m.	0	
11 h. 15 m.-11 h. 20 m.	0	

The subsequent injection of cocaine had no more effect than at first. Cocaine therefore causes no secretion from the submaxillary gland from sympathetic stimulation nor from direct action on the secretory cells. When it is injected while the chorda tympani is intact, (i.e. before atropine) it augments the pilocarpine secretion, as was shown in another experiment.

Thus in the sphere of influence of the cervical sympathetic system cocaine also departs from the adrenaline type, so that if the dilatation of the pupil under cocaine arises from sympathetic stimulation, the terminations in the iris alone of those examined respond to it. The grounds on which this view is founded are that the reaction is often (perhaps generally) absent after excision of the superior cervical ganglion, but in view of the more recent work on adrenaline, this argument cannot be regarded as conclusive. On the other hand the dilatation may arise from depressant action on the muscle directly, such as seen in the case of the vessels and heart. Or it is possible that there is a transient stimulation of the iris muscle such as occurs in the intestine, followed by depression, but the augmentor phase may be so slight as to pass unnoticed; in view of the strong contraction always present from oculomotor activity, it is not unlikely that such a slight increase as cocaine would cause by action on the muscle would escape observation.

Another feature, which has been ascribed to sympathetic stimulation is the constriction of the vessels of the conjunctiva on the local application of cocaine. This may however arise from direct action on the muscle of the vessels, the augmentor action of cocaine alone being elicited. It is true that I have not observed vasoconstriction in perfused vessels, nor indeed from vessels separated from the central nervous system, but this discrepancy may arise from some such factor as difference of concentration.

This action of cocaine in first stimulating and then depressing muscle is in harmony with the action on less differentiated forms of protoplasm, which was first examined by Sighicelli and Albertoni¹⁸ and which is now recognised to be analogous to its effects

¹⁸ Pflueger's Arch. f. Physiol., 1891, xlviii, p. 307.

on nerves and their sensory terminations. The changes in the iris are thus not an exception to its general action on living matter, but merely a further example of its tendency to first stimulate and then paralyse all protoplasm.

SUMMARY

1. The results of these experiments may be summarised in the following table.

ORGAN	EFFECT OF COCAINE	EFFECT OF SYMPATHETIC STIMULATION (ADRENALINE)
Surviving vessels of frog and mammal	Dilatation	Constriction
Vessels after destruction of central nervous system	None	Constriction
Heart (frog's)	Depressor	Augmentor
Heart (mammalian)	Depressor	Augmentor
Intestine	Augmentation (small dose)	Inhibition
	Depression (large dose)	
Stomach	Contraction	Inhibition
Uterus		
Rabbit's non-pregnant	Contraction, relaxation (large dose)	Contraction
Dog's non-pregnant	Contraction, relaxation	Contraction
Cat's non-pregnant	Contraction	Inhibition
Cat's pregnant	Contraction	Contraction
Bladder	Augmentation	Inhibition
Salivary gland	None	Secretion.

2. Cocaine therefore first increases the activity of unstriated muscle and then depresses it, whatever be the nature of the sympathetic control. In some instances the phase of increased activity was not observed.

3. It is argued that the dilation of the pupil under cocaine arises from direct action on the muscle of the iris and not from stimulation of the terminations of the sympathetic in the iris.

4. The action of cocaine in first stimulating and then paralysing muscle fibre (including that of the iris) is analogous to its effects on other forms of living matter, including that on sensory organs when directly applied to them.

THE BLOOD FLOW IN A PATIENT WITH DOUBLE AORTIC AND DOUBLE MITRAL DISEASE¹

L. H. NEWBURGH² AND J. H. MEANS³

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In connection with the studies of the blood flow recorded in the preceding paper, opportunity was afforded to make similar observations upon a patient with valvular heart disease.

The patient, C. L., an Italian man of 38 years and weighing 63 kgs., had had several attacks of poly-articular rheumatism. He had never had symptoms suggesting a definite attack of cardiac decompensation. He was able to do ordinary work without discomfort, but heavy pick and shovel work caused dyspnoea.

Physical examination showed a heart enlarged both transversely and downwards with a forcible impulse. Double murmurs at the aortic and mitral areas were heard. Slight cyanosis was present. No dyspnoea was observed. No edema or other evidence of chronic passive congestion was found.

Electrocardiography⁴ showed an aberrant P wave and a prolonged PR interval (the subject had had no digitalis). Occasional ventricular premature beats were found. There was no right or left ventricular preponderance.

Radiograph of the chest showed cardiac enlargement, especially to the right.

The Wasserman test on the blood was negative.

The oxygen carrying capacity of the blood was 173 cc. per litre, and the residual air was 1290 cc.

¹ From the medical service of the Massachusetts General Hospital. Expenses defrayed in part by a grant from the Council on Pharmacy and Chemistry of the American Medical Association, and in part by a grant from the Proctor Fund of the Harvard Medical School.

² Assistant visiting physician, Massachusetts General Hospital.

³ Henry P. Walcott Fellow in Clinical Medicine, Harvard University.

⁴ We are indebted to Dr. P. D. White for the electrocardiography.

The Krogh-Lindhard method was used to determine the blood flow and the experiments were planned in the same way as those described in the preceding paper. A series of resting experiments was secured first. The subject lay flat on the back for half an hour after which a respiration and blood flow experiment were carried out. The results are given in Table I.

The subject's reduced blood flow while at rest varied from 2.5 litres to 6.1 litres per minute; the average being 4.6 litres. The

TABLE I

Subject C. L.

Resting experiments lying flat on back

EXPERIMENT NUMBER	DATE	VALUES FOUND IN BLOOD FLOW EXP.					REDUCED VALUES				
		Oxygen cc. per min.	Blood flow litres per min.	Oxygen per litre of blood cc.	Pulse	Vol. per beat cc.	Oxygen cc. per min.	Blood flow litres per min.	Coefficient of utilization	Pulse	Vol. per beat cc.
		a	b	$\frac{a}{b} = c$	d	$\frac{b}{d} = e$	A	$b \frac{A}{a} = B$	C	D	$\frac{B}{D} = E$
	Jan.										
44A	15	449	3.31	135	100	33	343	2.53	78	72	35
44B	15	378	6.03	63	85	71	343	5.48	36	72	76
45A	18	359	5.65	64	—	—	252	3.96	37	68	58
45C	18	245	5.23	47	87	60	252	5.39	27	68	79
46A	22	329	5.74	57	79.5	72	247	4.30	33	76	57
46B	22	372	9.13	41	76.3	120	247	6.06	24	76	80
Average.....							281	4.62	39	72	64

systolic output or volume per beat varied from 35 cc. to 80 cc. Average 64 cc. The coefficient of utilization of the oxygen carrying capacity of the blood was from 24 per cent to 78 per cent.⁵ These results will be seen to be strikingly like those for the normal subjects J. H. M. and L. H. N.

A series of experiments showing the effect of increasing amounts of muscular work were also secured with the subject C. L. The results are given in Table II. From the values for blood flow

⁵ We should question the coefficient of 78 per cent obtained in experiment 44A. The range in all the other experiments is only from 24 per cent to 36 per cent.

and oxygen absorption given in the table, curves have been constructed and are shown in figure 1. In figure 2 a curve for the pulse rate has been plotted directly. A curve for the volume per beat has been constructed from the pulse rate and blood flow curves, and one for the coefficient, from the blood flow and oxygen curves; these two curves are shown in figure 2.

As with the normal subject, the oxygen absorption and blood flow show a parallel rise. The mechanism by which the blood

TABLE II
Subject C. L., Work Experiments

EXPERIMENT NUMBER	DATE	VALUES FOUND IN BLOOD FLOW EXP.			REDUCED VALUES					VENTILATION OF LUNGS LITRES PER MIN.	WORK AS RECORDED BY EROUMETER KG. METERS PER MIN.
		Oxygen cc. per min.	Blood flow litres per min.	Oxygen per litre of blood cc.	Oxygen cc. per min.	Blood flow litres per min.	Coefficient of utilization	Pulse	Vol. per beat cc.		
		a	b	$\frac{a}{b} = c$	A	$b \frac{A}{a} = B$	C	D	$\frac{B}{D} = E$		
	Mar.										
47	10	805	9.02	89	658	7.37	51	100	74	21.0	90
51	20	1112	12.85	87	798	9.22	50	100	92	17.4	216
48A*	12	916	15.17	60	935	15.48	35	96	161	24.4	248
49B*	12	901	11.65	77	935	12.06	44	96	126	24.4	248
49A	15	1156	11.32	102	1050	10.29	59	106	97	21.5	427
49B	15	1013	11.99	85	1050	12.40	49	106	117	21.5	427
50A	18	1234	13.81	89	1045	11.70	51	114	103	21.4	626
50B	18	1311	16.10	82	1045	12.81	47	114	112	21.4	626

* Not used in plotting curves.

Experiments enclosed thus } were averaged in plotting curves.

flow is increased is essentially the same as with the normal subject. In other words, as long as the supply of venous blood is "inadequate," the increase is met chiefly by an increase in volume per beat. After it becomes "adequate" (with this subject at about 400 kg. meters of work per minute) the increase is produced chiefly by an increase in pulse rate. The transition, or the point where presumably the supply becomes "adequate" occurs while the subject is doing less work than in the case of

J. H. M. The maximum systolic discharge, likewise, is not so great, being 107 cc. as against the normal subject's 118 cc.

The question of blood flow is of vast importance for a proper understanding of the circulation. The suggestion made by

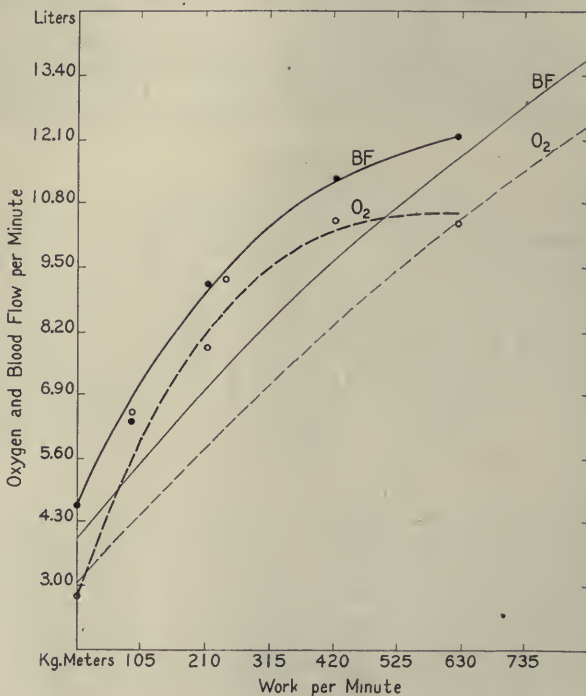


FIG. 1. Subject C. L. Blood flow BF shown by solid dots and line. Oxygen absorption, O₂, shown by circles and broken line. Plotted directly from results given in Table II.

NOTE: For oxygen, omit decimal point and read in ccs.

For comparison BF and O₂ of subject J. H. M. are also shown, but in lighter lines.

Boothby⁶ of the existence of a circulatory centre regulating the blood flow in a similar way to the regulation of pulmonary ventilation by the respiratory centre, is interesting. The essential function of pulmonary ventilation is the elimination of carbon

⁶ Boothby, W. M.: Determination of the circulation rate in man. *Am. Jour. Physiol.*, 1915, xxxvii, 383.

dioxide; that of blood flow the transportation of oxygen; or in other words the elimination of carbon dioxide bears the same relation to pulmonary ventilation as does the transportation of oxygen to the blood flow. Since the two processes are so analogous it seems reasonable that they should be regulated in a similar manner.

In regard to the question of pulmonary ventilation and carbon dioxide elimination plenty of data are available in the literature.⁷

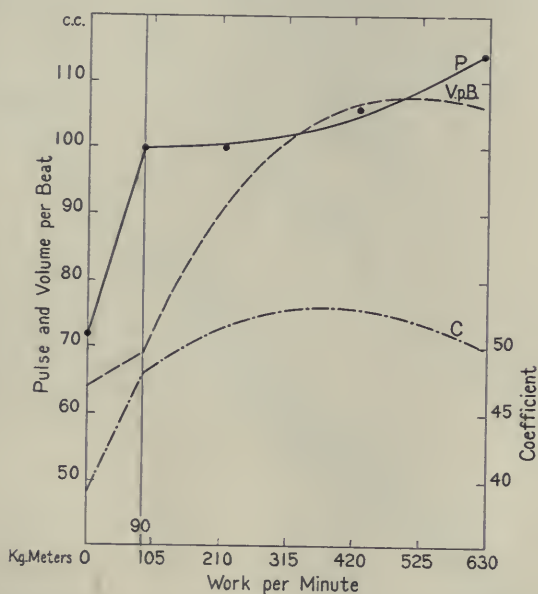


FIG. 2. Subject C. L. Pulse rate P, plotted directly from results given in Table II. Volume per beat V. p. B. and coefficient, C, constructed from curves BF and O_2 , figure 1, and curve C, figure 2.

In regard to blood flow, however, very few observations have been made. For the sake of comparison we have plotted the blood flows of C. L. and J. H. M. in terms of oxygen absorption and have shown them together with those of Boothby (for

⁷ See particularly Douglas and Haldane: *Journal of Physiology*, 1909, xxxviii, 420, and Campbell, Douglas, Haldane and Hobson: *Journal of Physiology*, 1913, xlv, 301.

himself) in figure 3. The curves for blood flow of the three subjects are very nearly coincident, which we believe is a fact of considerable importance in that it shows that the increase in

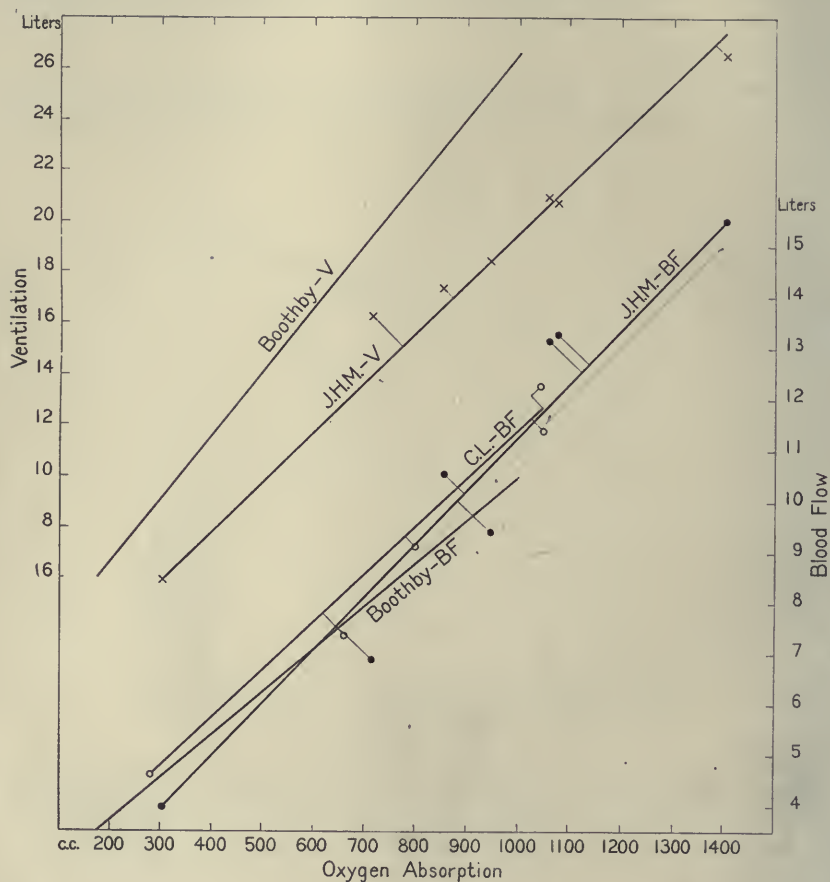


FIG. 3. Blood flow, BF, and pulmonary ventilation, V of J. H. M., C. L. and Boothby, plotted in terms of oxygen absorption.

blood flow is governed by the same law in different individuals, and is especially interesting since one of the three individuals observed had a badly damaged heart.

SUMMARY

Determinations of the blood flow during rest and in response to increasing amounts of muscular work in a patient with valvular heart disease are reported.

No essential differences between the cardiac and normal subject were found.

In both the normal and the cardiac subject the oxygen absorption and the blood flow showed a progressive rise with increasing amounts of work. This rise in blood flow was met at first chiefly by an increase in systolic output until a maximum was reached; after that by an increase in pulse rate.

THE EFFECT OF CAFFEINE UPON THE BLOOD FLOW IN NORMAL HUMAN SUBJECTS¹

J. H. MEANS² AND L. H. NEWBURGH³

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Since caffeine is a commonly employed therapeutic agent, an exact knowledge of its pharmacological action in human beings is a matter of extreme importance. In previous papers Newburgh⁴ has considered its effect upon blood pressure (in man), and Edsall and Means,⁵ and Higgens and Means,⁶ its effect upon respiration (in man). The present communication will deal with its effect upon the volume of the circulation, or, as it is more commonly called, the blood flow.

METHODS

Methods for determining the blood flow in man have been devised by a number of investigators, notably Bornstein,⁷

¹ From the medical service of the Massachusetts General Hospital. Expenses defrayed in part by a grant from the Council on Pharmacy and Chemistry of the American Medical Association, and in part by a grant from the Proctor Fund of the Harvard Medical School.

² Henry P. Walcott Fellow in Clinical Medicine, Harvard University.

³ Assistant visiting physician, Massachusetts General Hospital.

⁴ Newburgh, L. H.: Use of strychnine and caffeine as cardio-vascular stimulants in the acute infectious diseases. *Archives Internal Medicine*, 1915, xv, 458.

⁵ Edsall and Means: Effect of strychnine, caffeine, atropin, and camphor on the respiration and respiratory metabolism in normal human subjects. *Archives Internal Medicine*, 1914, xiv, 897.

⁶ Higgens and Means: Effect of certain drugs on respiration. *This Journal*, vii, 1, 1915.

⁷ Bornstein, A.: Eine Methode zur vergleichenden Messung des Herzschlagvolumens beim Menschen. *Archiv. f. die Gesamte Physiologie*, 1910, cxxxii, 307.

Markoff, Müller and Zuntz,⁸ Krogh and Lindhard,⁹ Plesch,¹⁰ and Haldane¹¹ and his co-workers. We have selected that of Krogh and Lindhard as being best suited to our needs. In brief the method consists in determining the rate of absorption of nitrous oxid gas in the lungs. Since nitrous oxid is a gas which forms no chemical combination with haemoglobin, but which goes into physical solution freely in blood plasma and, at body temperature, according to a definite coefficient, which has been determined,¹² it is possible to calculate how much blood must have passed through the lungs in order to absorb a measured amount of nitrous oxid from an original mixture of known concentration. A detailed description of the method here would be quite superfluous since it has been carefully described by the originators,¹³ and has also been subjected to careful scrutiny and criticism in a recent paper by Boothby.¹⁴

The blood flow experiment as carried out by us is precisely as done by Krogh and Lindhard. The blood flow was calculated by the shorter method described by these authors.¹⁵ The residual air determinations were made by their hydrogen method. The nitrous oxid analyses were made by combustion with pure hydrogen in the Haldane gas analysis apparatus. The apparatus used for the blood flow experiments consisted in a 6-litre spirometer and three way valve, both obtained from the workshop of Krogh's laboratory.

⁸ Markoff, Müller and Zuntz: Neue Methode zur Bestimmung der im menschlichen Körper umlaufenden Blutmenge. *Zeitschrift f. Balneologie*, 1911, iv, 373.

⁹ Krogh and Lindhard: Measurement of blood flow through lungs of man. *Skandinavisches Archiv f. Physiologie*, 1912, xxvii, 100.

¹⁰ Plesch, J.: Hämodynamische Studien. *Zeitschrift f. experimentelle Pathologie u. Therapie*, 1909, vi, 462.

¹¹ Christiansen, Douglas and Haldane: Absorption and dissociation of carbon dioxide by human blood. *Journal of Physiology*, 1914, xlviii, 263.

¹² As the coefficient of absorption of nitrous oxide in blood, we have used 0.43 which was determined by Siebeck (*Skandinavisches Archiv. f. Physiologie*, 1909, xxi, 368), for ox blood.

¹³ Krogh and Lindhard: *Loc. cit.*

¹⁴ Boothby, W. M.: Determination of circulation rate in man. *American Journal of Physiology*, 1915, xxxvii, 383.

¹⁵ Krogh and Lindhard: *Loc. cit.*

Determinations of the respiratory exchange were made just before the blood flow experiments by means of the Douglas respiration apparatus.¹⁶

The oxygen capacity of the blood of the two subjects was determined from their haemoglobin, Haldane having shown that the oxygen capacity is directly proportional to the haemoglobin determined colorimetrically.¹⁷ The haemoglobin was determined in the form of acid hematin by a Hellige colorimeter. With this instrument the color can be read within 1 per cent by different observers. The oxygen capacity of a normal subject was determined by the Barcroft differential blood gas apparatus¹⁸ and the haemoglobin read in the colorimeter at the same time. A capacity of 185 cc. per litre was taken as 100 per cent haemoglobin, and a factor for the colorimeter was obtained. Subsequently oxygen capacities were calculated from the haemoglobin according to the formula

$$\text{O}_2 \text{ Capacity} = \text{Hgb per cent} \times \frac{185}{100}$$

In the rest experiments and in some of the work experiments the pulse rate was obtained by a sphygmograph attached to the radial artery.

For the work experiments a bicycle ergometer of the type described by Martin was used.¹⁹

The general sequence of events in any experiment was as follows: In the case of the rest experiments the subject lay flat on his back on a couch for half an hour before any determinations were made. In the case of the work experiments he rode on the ergometer, at as nearly as possible an even rate, for half an hour. A respiration experiment (of about five minutes duration for the rest, and two for the work experiments) was then done

¹⁶ Douglas, C. G.: Method for determining the total respiratory exchange in man. *Journal of Physiology*, xlii, Proceedings, 1911, p. xvii.

¹⁷ Haldane and Smith: Mass and oxygen capacity of the blood in man. *Journal of Physiology*, 1900, xxv, 331.

¹⁸ Barcroft, J.: *Respiratory function of the blood*, 295.

¹⁹ Martin, C. J.: Convenient form of bicycle ergometer. *Journal of Physiology*, xlviii, Proceedings, p. xv, 1914.

with the Douglas apparatus. Following this, one to three blood flow experiments were carried out in the course of the next half hour.

FACTORS TO BE DISCUSSED

Blood flow. The total blood flow determined in the blood flow experiments is subject to a reduction as done by Krogh and Lindhard.²⁰ The mechanical conditions imposed upon the circulation by holding the breath vary in different experiments, because of the varying amounts of air enclosed in the chest. The absorption of nitrous oxid and oxygen are both determined in the blood flow experiment, and in addition that of oxygen is also determined in a respiration experiment. During the latter the conditions are essentially normal, so we may accept the oxygen absorption as the correct one. Any change in the absorption of nitrous oxid due to mechanical alterations in the blood flow experiment will similarly affect the oxygen absorption. So we can reduce the observed blood flow to the real blood flow by means of the oxygen, thus,

Blood flow observed : O_2 absorption found in blood flow experiment : : real blood flow : O_2 absorption found in respiration experiment.

The blood flow figures so obtained are termed reduced values. It must be borne in mind that the blood flow determined by this method is that through the lungs, or in other words, from the right to the left heart. Unless, however, there is a storage of blood in either the pulmonary or systemic circuits or a rapid change in the total volume of blood, the flow from the right to the left heart in a given time unit will equal that from the left to the right. Since in all these experiments every effort was made to have the subject in a state of circulatory equilibrium before the blood flow experiments were done, we feel justified in assuming that the flow through the lungs is equal to the output from the left ventricle in a given unit of time.

²⁰Krogh and Lindhard: Loc. cit.

Oxygen absorption. The values obtained from the respiration experiments are always taken as the true oxygen absorption.

Pulse rate. As the true pulse rate we have taken that obtained just before the blood flow experiments (often during the respiration experiments) while the subject was in a state of circulatory equilibrium. This seems more rational than using the pulse rate obtained during the blood flow experiment, since the work of making the respirations necessary for the blood flow experiment, together with a certain element of excitement, invariably causes a rise in pulse rate. During the work experiments after half an hour of riding the pulse rate counted either during a respiration experiment or while simply riding, was found to be remarkably constant.

Volume per beat or systolic discharge. As the volume per beat or systolic discharge we have taken the reduced blood flow per minute divided by the true pulse rate. In the tables this factor is given as the blood flow obtained in any experiment divided by the pulse rate found in that experiment. In the diagrams of the work experiments the blood flow, pulse, and oxygen absorption have been plotted for increasing amounts of work and the most probable curves drawn through the individual points. The curve for the volume per beat was constructed not from the values obtained in individual experiments, but from the blood flow and pulse rate curves.

The coefficient of utilization of the oxygen carrying capacity of the blood. The oxygen utilized per litre of blood was obtained by dividing the oxygen absorption found in the blood flow experiment by the unreduced blood flow. If the oxygen were 328 cc. and the blood flow were 4.5 litres per minute, we should have $\frac{328}{4.5} = 73$ cc. of oxygen utilized per litre of blood. Now if the subject's oxygen capacity were 193 cc. per litre the coefficient of utilization of the oxygen carrying capacity would be $\frac{73}{193} = 38$ per cent. In the tables the coefficient obtained in the individual experiments are given; in the diagrams a curve for the coefficient has been constructed from the blood flow and oxygen curves.

Ventilation of the lungs. The total ventilation of the lungs was determined by the respiration experiments and is given in the tables, also a curve showing the increase in ventilation with increasing amounts of muscular work with the subject J. H. M. is shown in figure 1.

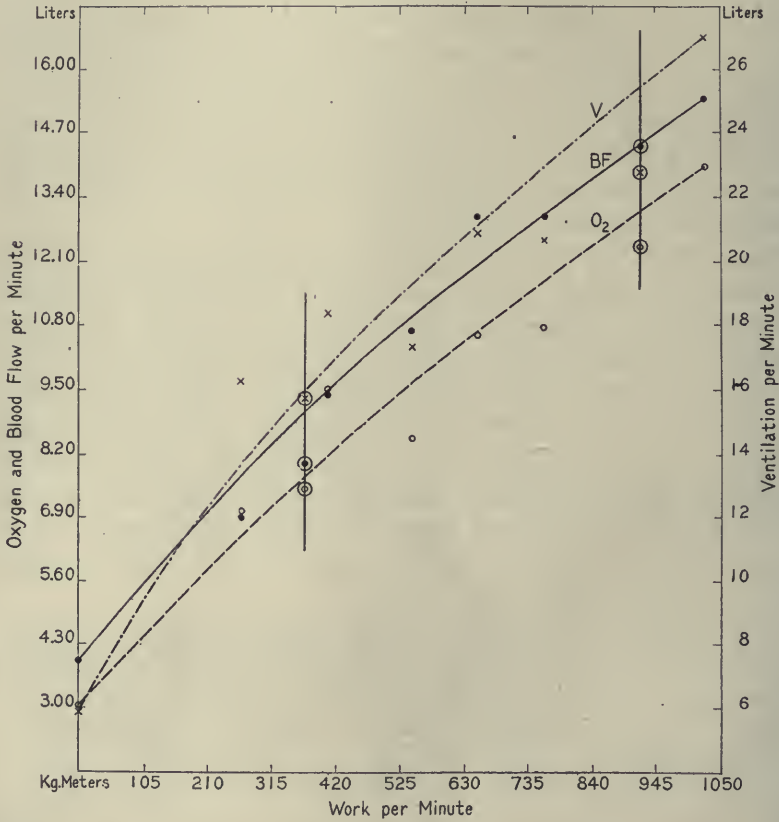


FIG. 1. Subject J. H. M. Effect of increasing amounts of muscular work. Total ventilation of lungs, V , shown by crosses. Blood flow, BF , by solid dots. Oxygen absorption, O_2 , by small circles. Plotted directly from results shown in Table VI.

The caffeine experiments are indicated by perpendicular lines and the individual factors by the same symbols as in normal experiments, but enclosed in circles.

NOTE. For oxygen omit decimal point and read in cc.

EXPERIMENTS PERFORMED

The general plan of the investigation was to determine the normal blood flow during rest and during work, and having determined this in any given individual, to see whether the administration of caffeine caused any change, other factors remaining equal.

The subjects were two in number. J. H. M. was studied during rest and work; L. H. N. during rest only. The statistics of the two subjects were as follows:

J. H. M. Male, 30 years of age, in good health. Occupation, physician. Weight 73 kg. Height 175 cm. Oxygen capacity 193 cc. per litre. Residual air, 1140 cc.

TABLE I

Preliminary experiments on subject J. H. M., lying flat

EXPERIMENT NUMBER	DATE	VALUES FOUND IN BLOOD FLOW EXPERIMENTS					REDUCED VALUES				
		Oxygen cc. per min.	Blood flow litres per min.	Oxygen per litre of blood cc.	Pulse	Vol. per beat cc.	Oxygen cc. per min.	Blood flow litres per min.	Coefficient	Pulse	Vol. per beat cc.
		a	b	$\frac{a}{b} = c$	d	$\frac{b}{d} = e$	A	$b \frac{A}{a} = B$	C	D	$\frac{B}{D} = E$
	Nov.										
9	2	290	5.8	50	83	70	(252)	5.0	26	—	—
11	4	258	5.0	52	79	63	(252)	4.9	27	—	—
12	4	206	4.5	46	72	62	(252)	5.5	24	—	—
13	6	210	4.3	49	55	78	(252)	5.2	25	—	—
14	8	311	5.8	54	61	95	(252)	4.7	28	—	—
15	9	261	3.6	72	71	51	(252)	3.5	37	—	—

L. H. N. Male, 32 years of age, in good health. Occupation, physician. Weight 61 kg. Height 169 cm. Oxygen capacity 173 cc. per litre. Residual air, 940 cc.

In Table I are shown a series of preliminary experiments upon the subject J. H. M. The results in this series are unreliable because of the fact that sufficient oxygen was not added to the original mixture in the recording spirometer, and consequently the oxygen in the second sample of alveolar air frequently fell

below 10 per cent. As a result the figures for oxygen absorption obtained in the blood flow experiment are too low and consequently the reduced blood flow is too high. In this series and also in the preliminary series on L. H. N. (see Table II), respiration experiments were not done for each blood flow experiment. An average figure for the subject's oxygen absorption at rest was assumed and the blood flow reduced to that. (Indicated in the tables by a parenthesis.)

TABLE II
Preliminary experiments on subject L. H. N., lying flat

EXPERIMENT NUMBER	DATE	VALUES FOUND IN BLOOD FLOW EXPERIMENTS					REDUCED VALUES				
		Oxygen cc. per min.	Blood flow litres per min.	Oxygen per litre of blood cc.	Pulse	Vol. per beat cc.	Oxygen cc. per min.	Blood flow litres per min.	Coefficient	Pulse	Vol. per beat cc.
		a	b	$\frac{a}{b} = c$	d	$\frac{b}{a} = e$	A	$b \frac{A}{a} = B$	C	D	$\frac{B}{D} = E$
1	Oct. 19	213	7.2	30	89	81	(222)	7.5	17	—	—
16	Nov. 9	222	7.7	29	82	94	(222)	7.7	17	—	—
19	12	232	7.1	33	80	89	(222)	6.8	19	—	—
20	16	234	9.1	26	100	91	(222)	8.6	15	—	—
26	23	201	5.7	35	84	68	(222)	6.3	20	76	83
27	24	271	7.2	38	92	78	(222)	5.9	22	82	72
34	Dec. 11	252	5.1	49	80	64	(240)	4.9	28	70	70
35	11	236	4.8	49	76	63	(240)	4.9	28	68	72
36	15	219	5.2	42	73	71	(240)	5.7	24	68	84
37A	16	297	5.2	57	95	55	259	4.5	33	72	62

The high values for the blood flow in the preliminary experiments on the subject L. H. N. were in part due to low oxygen in the original mixture, and in part because a fifteen minute period of rest was used instead of a half-hour as in all the later experiments. Fifteen minutes was not long enough to get this subject into circulatory equilibrium.

In Tables III and IV are shown series of experiments on both J. H. M. and L. H. N. during rest, in which the above mentioned

TABLE III

Subject J. H. M. Resting experiments lying flat on back

EXPERIMENT NUMBER	DATE	VALUES FOUND IN BLOOD FLOW EXPERIMENTS					REDUCED VALUES					VENTILATION OF LUNGS LITRES PER MIN.
		Oxygen cc. per min.	Blood flow litres per min.	Oxygen per litre of blood cc.	Pulse	Vol. per beat cc.	Oxygen cc. per min.	Blood flow litres per min.	Coefficient of utilization	Pulse	Vol. per beat cc.	
		a	b	$\frac{a}{b} = c$	d	$\frac{b}{d} = e$	A	$b \frac{A}{a} = B$	C	D	$\frac{B}{D} = E$	
23A	Dec. 23	330	3.56	92.6	74	48.1	288	3.10	48	70	44.3	5.32
38B*	23	390	3.92	99.5	82.6	47.4	288	2.90	52	72	40.2	
38C	23	374	4.87	76.8	59.6	81.6	314	4.09	40	62.5	65.4	5.98
38D*	23	554	8.22	67.4	84.9	96.9	314	4.66	35	70	66.6	
42A	Jan. 6	329	4.78	68.8	74	64.6	332	4.83	36	72.5	66.6	5.73
52	Mar. 28	437	6.42	68.1	—	—	287	4.22	35	65.0	65	6.42
Average.....							304	3.97	41	69	57	5.9

* These two experiments were done by the residual method.

TABLE IV

Subject L. H. N. Resting experiments lying flat on back

EXPERIMENT NUMBER	DATE	VALUES FOUND IN BLOOD FLOW EXPERIMENTS					REDUCED VALUES					VENTILATION OF LUNGS LITRES PER MIN.
		Oxygen cc. per min.	Blood flow litres per min.	Oxygen per litre of blood cc.	Pulse	Vol. per beat cc.	Oxygen cc. per min.	Blood flow litres per min.	Coefficient of utilization	Pulse	Vol. per beat cc.	
		a	b	$\frac{a}{b} = c$	d	$\frac{b}{d} = e$	A	$b \frac{A}{a} = B$	C	D	$\frac{B}{D} = E$	
39A	Dec. 23	418	7.59	55	89.5	84	248	4.50	* 32	78	57.7	5.2
39C	23	381	6.03	63	68.5	83	237	3.75	36	65	57.7	4.6
39D	23	444	8.68	51	73	119	237	4.64	29	66	70.3	
40A	Jan. 1	496	8.95	56	104	86	(240)	4.32	32	82	53.8	—
40B	1	322	5.81	55	86	68	(240)	4.34	32	74	58.5	—
43A	7	318	6.88	46	84	82	257	5.56	27	85	65.5	5.8
Average.....							243	4.52	31	75	60.6	5.2

TABLE V

Caffeine experiments on both subjects

NOTE—The caffeine was given with equal parts of sodium salicylate. The dosage given below is in terms of pure caffeine

EXPERIMENT NUMBER	DATE	SUBJECT	VALUES FOUND IN BLOOD FLOW EXPERIMENTS				REDUCED VALUES					WORK AS RECORDED BY ERGOMETER KG.	VENTILATION OF LUNGS LITRES PER MIN.		
			Oxygen cc. per min.	Blood flow litres per min.	$\frac{a}{b} = \frac{c}{d}$ of blood cc.	Pulse	Vol. per beat cc.	Oxygen cc. per min.	$\frac{A}{B} = \frac{B}{D}$ per min.	Coefficient of Utilization	Pulse				$\frac{B}{D} = \frac{E}{D}$ Vol. per beat cc.
31A	Dec. 6	L. H. N.	260	6.97	37	89	78	(240)	6.44	21	83	77	0	0	30 min. after caffeine gr. v
31B	Dec. 6	L. H. N.	197	4.50	44	75	60	(240)	5.48	25	60	91	0	0	60 min. after caffeine gr. v
37A	Dec. 16	L. H. N.	246	5.93	41	84	71	231	5.57	24	71	78	0	0	40 min. after caffeine gr. vii ss
37B	Dec. 16	L. H. N.	199	4.45	45	84	53	245	5.48	26	76	72	0	0	80 min. after caffeine gr. vii ss
43A	Jan. 8	L. H. N.	318	6.88	46	84	82	257	5.56	27	85	65.5	0	0	Normal conditions. Done just before caffeine injection
43B	Jan. 8	L. H. N.	310	5.40	57	68	79	261	4.55	33	70	65	0	0	25 min. after caffeine gr. v
43C	Jan. 8	L. H. N.	426	6.77	63	71	95	261	4.15	36	62	67	0	0	65 min. after caffeine gr. v
42A	Jan. 6	J. H. M.	329	4.78	69	74	65	332	4.83	36	72	67	0	0	Normal conditions. Done just before caffeine injection
42B	Jan. 6	J. H. M.	350	8.67	40	80	108	329	8.15	21	70	116	0	0	35 min. after caffeine gr. v
42C	Jan. 6	J. H. M.	389	7.55	51	78	97	329	6.40	27	70	91	0	0	55 min. after caffeine gr. v
W9A	Mar. 8	J. H. M.	869	10.50	83	—	—	752	9.12	43	106	86	372	372	20 min. after caffeine gr. v
W9B	Mar. 8	J. H. M.	1090	10.27	106	—	—	752	7.09	55	106	67	372	372	30 min. after caffeine gr. v
W10A	Mar. 23	J. H. M.	1476	19.35	76	—	—	1242	16.30	39	124	131	916	916	20 min. after caffeine gr. v
W10B	Mar. 23	J. H. M.	1644	21.50	77	—	—	1242	16.25	40	128	127	916	916	30 min. after caffeine gr. v
W10C	Mar. 23	J. H. M.	1364	12.55	109	—	—	1242	11.40	56	128	89	916	916	40 min. after caffeine gr. v

sources of error were eliminated, the subject always rested for a half-hour or more before the experiment was done, and in which a half litre of oxygen was always added to the original mixture. In these two series, moreover, a respiration experiment was nearly always done.

The caffeine experiments during rest were carried out in the same way. After a preliminary rest flat on the back of thirty minutes duration, or more, caffeine sodium salisylate was injected subcutaneously. In about twenty-five minutes the respi-

TABLE VI

Subject J. H. M. Work experiments

The experiments enclosed thus } were averaged in plotting curves shown in figures 1 and 2

EXPERIMENT NUMBER	DATE	VALUES FOUND IN BLOOD FLOW EXPERIMENTS					REDUCED VALUES					VENTILATION OF LUNGS LITRES PER MIN.	WORK AS RECORDED BY PNEUMETER KG. METERS PER MIN.
		Oxygen cc. per min.	Blood flow litres per min.	Oxygen per litre of blood cc.	Pulse	Vol. per beat cc.	Oxygen cc. per min.	Blood flow litres per min.	Coefficient of utilization	Pulse	Vol. per beat cc.		
		a	b	$\frac{a}{b} = c$	d	$\frac{b}{d} = e$	A	$a \frac{A}{a} = B$	C	D	$\frac{B}{D} = E$		
W2	Feb. 13	1027	9.92	103	100	99	713	6.89	53	100	69	16.3	270
W8	Feb. 25	1084	10.77	101	—	—	947	9.41	52	99	95	18.4	410
W5A	Feb. 19	753	8.23	91	104	79	852	9.31	47	100	93	17.3	544
W5B	Feb. 19	982	13.61	72	102	133	852	11.80	37	95	124	17.3	544
W6A	Feb. 22	390	13.04	76	114	114	1060	13.98	39	110	127	20.9	653
W6B	Feb. 22	1178	13.65	86	112	122	1060	12.30	45	110	112	20.9	653
W4A	Feb. 17	1200	16.77	72	118	142	1075	15.00	37	111	135	20.7	760
W4B	Feb. 17	1640	17.60	93	107	164	1075	11.52	48	111	104	20.7	760
W7A	Feb. 25	1260	15.15	83	140	108	1404	16.86	43	140	120	26.5	1024
W7B	Feb. 25	1300	14.50	90	—	—	1404	15.63	47	140	112	26.5	1024
W7C	Feb. 25	1450	14.45	100	136	106	1404	14.00	52	130	108	26.5	1024

ration experiment was done and following it two blood flow experiments at intervals of twenty to forty minutes. The results of the caffeine experiments on the two subjects are collected in Table V.

To determine the action of caffeine during work we first of all made a series of "normal" experiments upon the subject J. H. M. under conditions of increasing amounts of muscular work.

The results are given in Table VI and in the form of curves in figures 1 and 2.

After these normal values had been determined, two caffeine experiments were done; one during light work, one during heavy. The results are given in Table V and are indicated in the two figures.

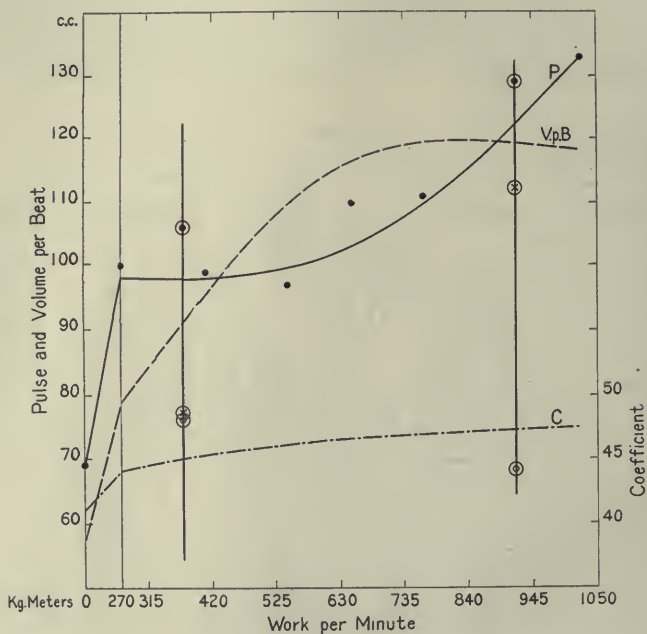


FIG. 2. Subject J. H. M. Effect of increasing amounts of muscular work. Pulse rate, *P*, shown by solid dots. Plotted directly from results shown in Table VI. Volume per beat, *V.p.B.*, and coefficient of oxygen capacity of the blood *C*, were constructed from curves *BF* and O_2 , figure 1, and curve *P*, this figure.

The caffeine experiments are indicated as in figure 1. Volume per beat by crosses and coefficient by small circles.

DISCUSSION OF RESULTS

The results of the resting experiments show a general agreement with those of Krogh and Lindhard. The reduced blood flow of J. H. M. varies from 2.9 to 4.8 litres per minute with an average of 4.0 litres. This marked variation in the blood flow

during rest, was also found by Krogh and Lindhard,²¹ and is undoubtedly due to the fact that the supply of blood during rest is, as Krogh suggests, "inadequate," that is, inadequate to fill the ventricles to their utmost capacity during each diastole.²² Under such circumstances the output of the heart is dependent upon the supply of venous blood and is not directly related to the functional capacity of the heart.

While the subject is doing any constant amount of work, however, the blood flow shows considerably less variation. The explanation of this suggested by Krogh, and which appeals to us as most probable, is that under conditions of work the supply of venous blood becomes "adequate."

The volume per beat or systolic output of the subject J. H. M. during rest is from 40 cc. to 67 cc. (average 57 cc.). The coefficient of utilization of the oxygen carrying capacity is from 35 to 52 per cent (average 41 per cent).

With the subject L. H. N. the resting blood flow varies from 3.7 to 5.6 litres (average 4.5 litres). The volume per beat is from 54 to 70 cc. (average 61 cc.). The coefficient is from 27 per cent to 36 per cent (average 31 per cent).

These results are in general quite similar to those obtained by Lindhard²³ in a series of normal persons.

The caffeine experiments during rest show some interesting results. In one experiment (42 A-C, Table V) with J. H. M. thirty-five minutes after 5 grains of caffeine the total blood flow rose to 8.15 litres, and fifty-five minutes after the injection it was still 6.4 litres. The blood flow immediately before the injection had been 4.8 litres and the subject's average resting blood flow was 4.0 litres, and the highest value ever obtained at rest without any drug 4.8 litres. The absorption of oxygen showed no increase after the caffeine, consequently there was a fall in the coefficient of utilization of the oxygen carrying capacity;

²¹ Krogh and Lindhard: Loc. cit.

²² Krogh, A.: Influence of venous supply upon the output of the heart. *Skandinavisches Archiv. f. Physiologie*, 1912, xxvii, 126.

²³ Lindhard, J.: Concerning the influence of ultraviolet light on the circulation in man. *Skandinavisches Archiv. f. Physiologie*, 1913, xxx, 73.

just before the caffeine injection it had been 36 per cent; thirty-five minutes after it was 21 per cent. The pulse rate showed no rise after the caffeine and hence the volume per beat showed a marked rise from 67 cc. before to 116 cc. after. The total ventilation of the lungs showed a rise from 5.7 litres before to 6.9 litres after the caffeine, this effect being probably due to a stimulation of the respiratory centre such as that found by Higgens and Means.²⁴

With L. H. N. in experiment 31 (Table V) half an hour after 5 grains of caffeine a blood flow of 6.4 litres was found and an hour later 5.5 litres. The subject's average resting value under normal conditions is 4.5 and the maximum 5.6. A reduction in the coefficient to 21 per cent, the subject's normal average being 31 per cent, and a rise in the volume per beat likewise occurred. In the second caffeine experiment with this subject (37 A-B, Table V) the same action was found but to a lesser degree. In forty minutes after $7\frac{1}{2}$ grains of caffeine the blood flow was 5.6 litres, the coefficient 24 per cent and the volume per beat 78 cc. In the third experiment with L. H. N. (43 A-C) no rise in blood flow or volume per beat occurred. In fact after the caffeine the blood flow was actually lower than the value found just before the injection. It is only fair to point out that this "normal" flow was the highest ever obtained with this subject.

At rest then, or in other words, when the supply of venous blood is "inadequate," caffeine frequently, though not invariably causes a rise in the total blood flow without a corresponding increase in the oxygen absorption, and hence a fall in the coefficient of utilization of the oxygen carrying capacity of the blood. Moreover, and this we believe is a point of extreme interest, the rise in blood flow occurs without any corresponding rise in pulse rate. Hence the systolic discharge is increased. This increase in the blood flow, without a rise in pulse rate, suggests to our minds that under these circumstances the action of caffeine is upon the supply of venous blood rather than upon the heart.

²⁴ Higgens and Means: Loc. cit.

²⁵ Boothby: Loc. cit.

Boothby²⁵ has suggested that there is a circulatory centre exercising a similar control over the blood flow to that of the respiratory centre over the ventilation of the lungs. The action of caffeine here noted might be a stimulation of such a centre, and this supposition is not unreasonable in view of the fact that caffeine has been shown to stimulate the respiratory centre.²⁶

During work under normal conditions the subject J. H. M. shows a steady increase in oxygen absorption, blood flow and pulmonary ventilation. The blood flow rises from the resting average of 4.0 litres up to about 15 litres when doing 1000 kg. meters of work per minute, or an increase of 280 per cent. The oxygen absorption rises from the resting average of 304 cc. to about 1380 cc. or an increase of 350 per cent. The ventilation of the lungs rises from the resting average of 5.9 litres to about 27 litres, or an increase of 360 per cent.

The mechanism by which the demand for an increased blood supply is met is interesting. Up to about 640 kg. meters per minute the increase is met at first entirely, later chiefly, by an increase in the systolic output, beyond that point at first chiefly and finally entirely, by an increase in pulse rate. According to our understanding of Krogh's theory of "adequate" and "inadequate" supply we should interpret this transition as meaning that the supply of venous blood in this subject gradually increases until at approximately 735 kg. meters of work it is entirely adequate; beyond that point, therefore, blood flow is a function of pulse rate alone.

The coefficient of utilization of the oxygen capacity shows a slight rise during work, from a resting average of 41 per cent to an average of 46 per cent during work. The importance of the coefficient is that it is an index of what may be called the economy of the circulation. It would be of no consequence were it not known to vary. Krogh and Lindhard,²⁷ however, found that in J. L., who may be considered an athlete, the coefficient rose very markedly during work, whereas in A. K., who is not an athlete, it rose very little. The phenomenon is of vast importance. It

²⁶ Higgens and Means: Loc. cit.

²⁷ Krogh and Lindhard: Loc. cit.

means that a well trained subject can transport a given amount of oxygen to his tissues with a much smaller blood flow than can the untrained. In other words, the circulation works to better economy. The saving to the heart must be enormous.

The effect of 5 grains of caffeine subcutaneously was tried twice with the same subject (J. H. M.) during work, once while doing 372 kg. meters per minute, once while doing 916 kg. meters. The values found for the blood flow and oxygen absorption fall easily within the limits of experimental error and normal variation. Further, no increase in ventilation, indicating an action on the respiratory center, was found. In fact, in the experiment done at 916 kg. meters there was even a decrease. The pulse rate may have been slightly increased and the volume per beat consequently reduced.

SUMMARY AND CONCLUSIONS

Experiments upon the blood flow of two normal subjects during rest, and of one subject during muscular work, are reported.

The action of caffeine on the blood flow was studied in both subjects while at rest, and in one during work.

The average blood flow of the two subjects at rest was 4.5 and 4.0 litres per minute; the systolic outputs were 61 and 57 cc.; the coefficients of utilization of the oxygen carrying capacity of the blood were 31 per cent and 41 per cent.

With increasing work a steady rise in blood flow, oxygen absorption and pulmonary ventilation was found. The increase in blood flow was produced first by an increase in systolic output until a maximum of 118 cc. was reached, beyond that by an increase in pulse rate. This suggested that the supply of venous blood in this subject becomes "adequate" at about 640 kg. meters of work per minute. The coefficient of utilization showed a slight rise during work indicating a slightly greater economy of the circulation.

After giving caffeine during rest, or when the supply of venous blood is "inadequate," evidence of drug action was found with

both subjects. This action consisted in an increase in total blood flow without a corresponding increase in oxygen absorption, and hence a decreased coefficient of utilization of the oxygen carrying capacity of the blood. The pulse rate was unchanged. Consequently the systolic output was increased.

During work probably no other action was obtained from caffeine than possibly an increase in pulse rate, and consequently slight diminution in systolic output.

It is suggested that during rest when the supply of blood to the right heart is "inadequate" caffeine increases the blood flow by increasing the venous supply through an action upon some mechanism outside the heart. When the supply becomes "adequate" or approaches adequacy, no such action is obtained.

Our sincere thanks are due to Dr. August Krogh both for instruction in the method, and for supplying the necessary apparatus.

AN APPARATUS FOR THE PERFUSION OF ISOLATED ORGANS

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The apparatus described in the following pages was designed for the purpose of maintaining a circulation of hirudinized or defibrinated blood through the vessels of an isolated surviving mammalian organ in a manner which should approximate as closely as possible to that obtaining in the living animal: it was further intended to permit the accurate measurement and independent adjustment of the mechanical factors of the circulation. It consists essentially of three parts, the pumps, the driving apparatus and an oxygenating device.

The pumps. Two pumps of identical size and construction are used, one for pumping blood through the organ under investigation, the other for pumping the blood through the oxygenating device. In this description the former will be referred to as the perfusion pump, the latter as the oxygenating pump. Each consists of a glass cylinder (fig. 1, 1) with a glass piston (2) ground to fit. Two small glass tubes are sealed in at the lower end and are bent so that they project parallel with each other in a plane at right angles to the cylinder. One is the inlet (3), the other the outlet (4) of the pump. On the outlet tube a glass stop-cock tube (5) is sealed in at right angles so that it stands parallel with the cylinder. This is called the filling tube.

The piston consists of a glass tube, sealed at one end, and accurately ground to fit the cylinder. A brass rod (6), threaded at one end, is set into it with plaster of paris so that the threaded end projects about half an inch above the upper end of the piston.

The cylinders have been constructed in triplicate in three sizes, viz., 10, 15 and 20 mm. inside diameter: all have the same length, 10.5 cm. Our experience thus far has been chiefly with those of the smallest size.

The cylinder and piston described is capable of pumping fluid in one direction against resistance only when supplied with competent valves. After much experience we adopted a method of alternate compression of the two rubber tubes (7, 8) which

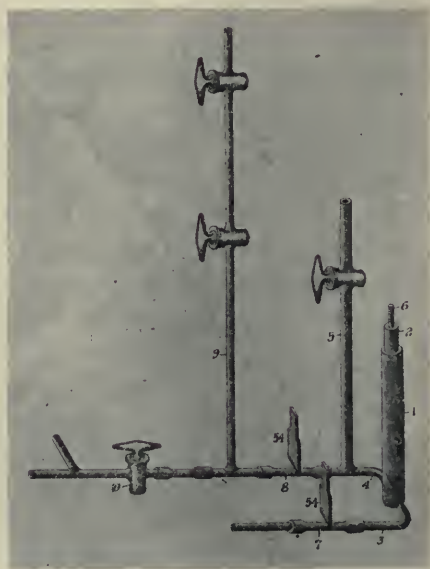


FIG. 1. Glass perfusion pump with connections for modifying character of pulse ($\times \frac{1}{2}$).

are attached to the glass inlet and outlet tubes of the cylinder. This is secured by the action of two cams to be described below. In actual use, when the piston of the pump rises the inlet of the pump is open, the outlet closed: the reverse is true when it falls.

Glass was chosen as the material for the pumps in order to avoid complications which might result from contact of blood with metal or other materials. The glass pumps are surprisingly durable, such

losses as we have sustained having arisen almost wholly from spontaneous breaks due to imperfect annealing.

Our experience with glass valves is possibly of interest. For some time we worked with a pair of valves attached to each cylinder, of the type shown in figure 2. Each valve consisted of a small glass chamber containing a glass swimmer loaded with mercury. The approximating surfaces of chamber and swimmer were ground so that when properly seated the valve was competent. For two reasons such a valve proved unsatisfactory: First, only under the most favorable circumstances does it seat so perfectly as wholly to prevent back flow. This makes the output of the pump vary under conditions which are apparently uniform. Second, when using hirudinized blood, clots formed soonest in the course of an experiment about the swimmer of the valve, apparently from its churning action. The smallest clot between its seating surfaces obviously renders such a valve partially or wholly incompetent.

The driving apparatus. Under this name is described the stand in which the pumps are held, and the mechanism for transmitting a thrust to the piston.

The stand consists of a heavy brass base (fig. 3, 11), shaped like the letter H. Its outside dimensions are: length, 10 inches; width, $9\frac{5}{8}$ inches; thickness, 1 inch. Into it are set four uprights of square brass: the two (12) which are set at the ends of the cross bar of the H being $\frac{1}{2}$ inch square and 21 inches high; the other two (13) are $\frac{3}{4}$ inch square and nearly 20 inches high. For the sake of firmness, the ends of each pair are joined by a cross piece.

About 4 inches above the base, a brass bracket (14) is securely fastened to each inner face of the uprights (12). Each bracket has a hole in it, $\frac{3}{8}$ inch in diameter, into which is tightly inserted a small rubber stopper (15) so that the upper surface of the stopper is $\frac{1}{8}$ inch above the upper face of the bracket. The bracket and stopper serve as a cushioned base upon which the lower end of the glass pump rests when fastened in position. (The two unnumbered short upright posts shown in figure 3 are makeshift additions to prevent vibration of the bracket.)



FIG. 2.

Sliding on the upright (12), above the bracket, is an adjustable brass cylinder or sleeve (16) which can be securely fastened at any point by the set-screw (17). Its purpose is to hold the glass cylinder firmly in an upright position on the bracket (14).

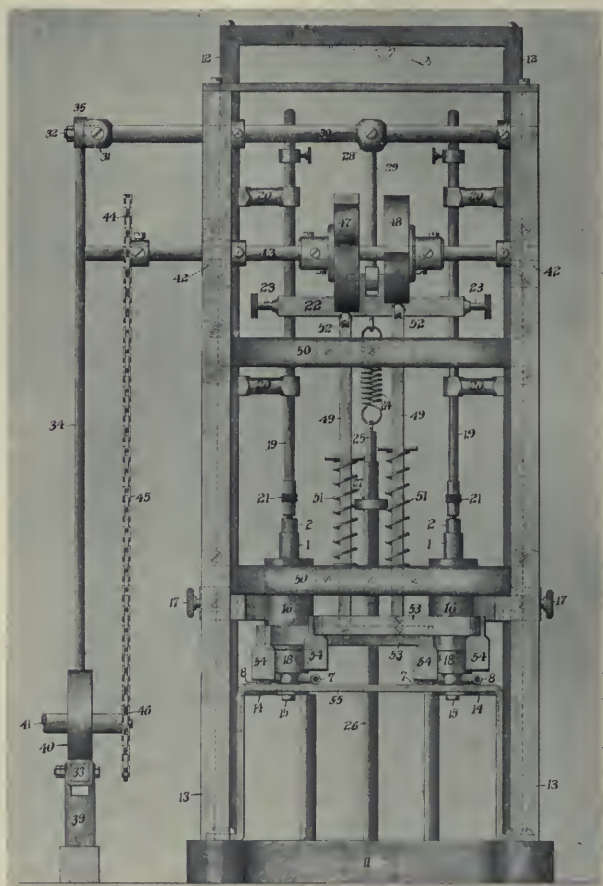


FIG. 3. Front elevation of stand and driving apparatus ($\times \frac{1}{5}$).

To protect the glass cylinder when it is held down by the brass sleeve (16) a short section of heavy rubber tubing (18), to act as a cushion, is slipped down over the glass cylinder before it is placed in the sleeve. The inner diameter of the sleeve is 25

mm., i.e., sufficient to admit the glass cylinder of largest size. Two brass bushings are made for each sleeve for use with the smaller glass cylinders.

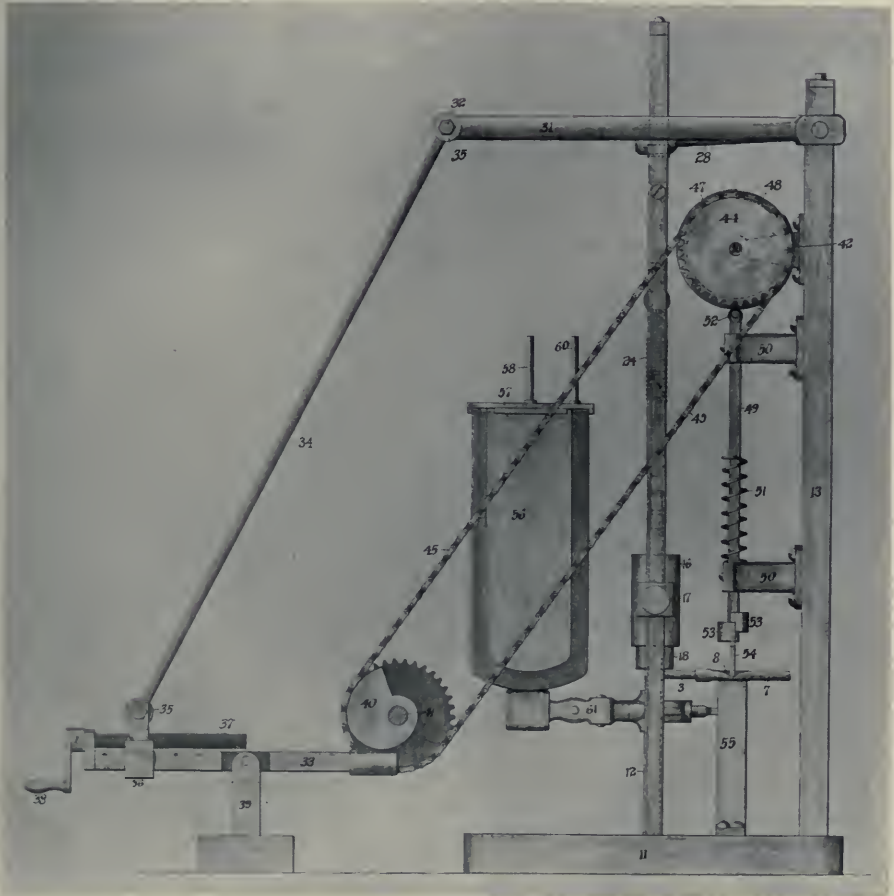


FIG. 4. Side elevation of stand and driving apparatus ($\times \frac{1}{2}$). The positions of support (39) and shaft (41) are incorrectly shown: they should be such as to make the position of the connecting rod (34) vertical.

Firmly screwed to each of the uprights (12) above the sleeve (16) are two additional sleeves (20, 20) 5 inches apart, the lower $11\frac{3}{4}$ inches from the base. Through these sleeves, the

inner diameter of which is just sufficient to admit it, passes a round brass rod (19), $\frac{1}{4}$ inch in diameter and threaded at the lower end. A small brass union (21) permits it to be firmly attached to the threaded end of the brass rod set into the piston of the pump. The up and down thrust of the piston is secured by joining it in this fashion to the brass rod (19) and attaching the latter to the cross head (22) by the set screw (23). The movements of the driving cam are transmitted to this cross head. It is essential that the centres of the brass sleeves (16, 20, 20) be in accurate vertical alignment.

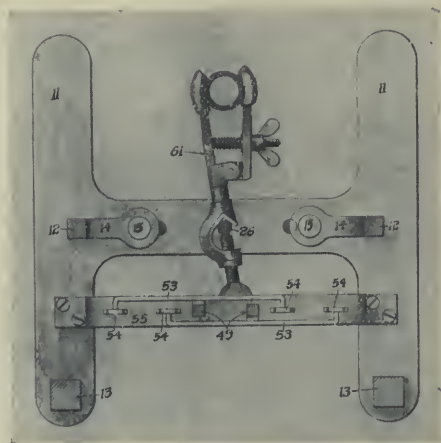


FIG. 5. Sectional diagram of stand ($\times \frac{1}{5}$).

The cross head (22) has a brass hook on its lower face through which passes the end of the strong steel spring (24). The other end of the spring is fastened to a slender brass rod (25) which slides in a hollow upright (26) mounted in the base. It can be fixed in any position by the set screw (27) and thus the tension of the spring, which is the motive force for the down stroke of the pump, can be varied at will. To the upper face of the cross head (22) is pivoted one end of a bent oscillating arm (28, 29), the other end of which is firmly screwed to the shaft (30) which passes through it. This shaft is mounted on the uprights (13, 13), $18\frac{3}{4}$ inches from the base. The projecting end of this shaft passes

into and is screwed to the end of the steel driving arm (31), 10 inches long, $\frac{5}{8}$ inch thick. Into the other end of the driving arm a steel peg (32) is inserted horizontally and at right angles with it. The upward movement of this driving arm gives the upward thrust to the piston, transmitted by the shaft (30), bent arm (28, 29) and cross head (22): the upward thrust is exerted against the tension of the spring (24).

The upward movement of the driving arm (31) is transmitted to it by the driving beam (33) to which it is attached by the connecting rod (34), $17\frac{1}{2}$ inches long. To each end of the connecting rod is screwed a brass bearing: that at the upper end (35) slips over the steel peg (32) and is held in place by a nut. Through the bearing at the lower end (35, figure 4) passes a steel pivot, by which the rod is attached to the rider (36) on the driving beam (33). The position of the rider on the driving beam can be adjusted by turning the screw (37), which passes through the rider, by means of the crank (38). The length of the thrust of the pump is thus variable within wide limits. A scale of forty divisions is etched on the outer surface of the driving beam, the length of each division being equal to the movement of the rider produced by one turn of the screw. We refer to thrust of the piston in terms of figures on this scale.

The driving beam (33) is made of $\frac{1}{2}$ inch square steel, 8 inches long. It is pivoted to the support (39) which is securely fastened to the table upon which the machine stands when in use. This support is so placed on the table that the end of the driving beam furthest from the rider is vertically below the centre of the driving cam (40), mounted on shaft (41) supported on the same table and driven by a pulley belted to an eighth-horse power motor. The end of the driving beam is held in firm contact with the cam by the tension of the spring (24), transmitted by the system of levers described: the rotation of the cam gives an up and down movement to the end of the driving beam and hence to the piston of the pump. By suitable gearing and by the introduction of a rheostat, the driving cam can be given any speed between 60 and 250 revolutions per minute.

It will be obvious from this description and the accompanying diagrams that the only work performed by the driving cam is the upstroke of the piston; its downstroke is the result of the tension of the spring (24). Hence only the number of strokes per minute depends upon the speed with which the cam revolves. The power of each stroke depends on the tension of the spring; the volume of each stroke depends upon the position of rider (36) on the driving beam. Each of the three factors, rate per minute, power and volume per stroke is independently adjustable and each may be altered while the machine is running without interruption.

The valve mechanism. The mechanism for alternately compressing the two rubber tubes which are attached to the inlet and outlet tubes of the glass pump is as follows: By means of two projecting supports (42) a $\frac{3}{8}$ -inch shaft (43) is mounted on the uprights (13, 13), $15\frac{1}{2}$ inches above the base.

The shaft projects on one side for several inches beyond its support, and on the projecting end is mounted a small sprocket wheel (44), $2\frac{5}{8}$ inches in diameter. By a chain belt (45) this wheel is belted to an identical sprocket wheel (46) mounted on the end of the driving cam shaft (41). Midway between its two supports, this shaft (43) carries two cams, $\frac{3}{4}$ inch apart. Their shapes and their relations to each other and to the driving cam are shown in figure 6. They are cut so that their revolution imparts a vertical movement of $\frac{1}{4}$ inch to each of the two rods (49, 49). These rods, actuated by the cams, alternately compress and release the rubber tubes (7, 8) attached to the inlet and outlet tubes of the glass pump. Each is made of brass, $\frac{5}{16}$ inch square, $9\frac{1}{4}$ inches long, and slides in the two supports (50, 50), mounted on the uprights (13, 13). The spiral steel springs (51, 51) lift the rods so that each is held firmly against its cam. At the upper end of each is pivoted a small steel roller (52) to reduce friction between cam and rod. At the lower end of each rod is fastened a horizontal arm (53) extending 2 inches on each side of the rod, to each end of which is fastened a spatula-shaped projection (54). A rigid band (55) made of $\frac{3}{16}$ -inch brass, $\frac{3}{4}$ inch wide, and bent so that its horizontal portion

is $4\frac{1}{8}$ inches above the base, is screwed at each end to the base in such a position that it forms a shelf over which the rubber tubes (7, 8) cross. Against this shelf, the tubes are compressed by the blunted edges of the two pairs of spatulate projections above mentioned: one pair compresses simultaneously the outlet tubes of the two pumps which the stand holds, the other pair compresses the inlet tubes.

The effectiveness of the entire apparatus as a pump depends upon the shape of the three cams employed and their proper timing. The chain gear between the driving cam shaft (41)

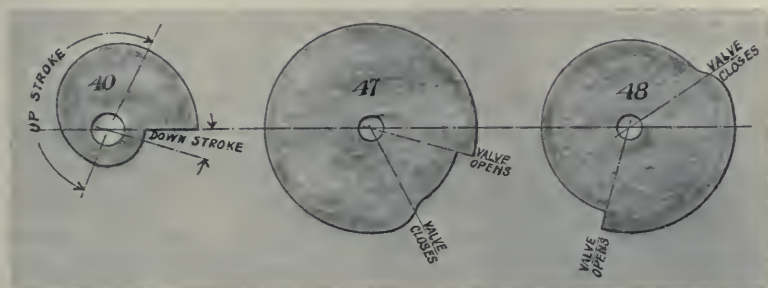


FIG. 6. Driving cam (40); outlet valve cam (47); inlet valve cam (48) ($\times \frac{1}{3}$).

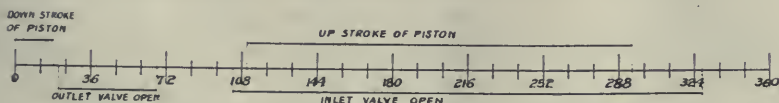


FIG. 7. Diagram showing relation of pump to valves during one complete cycle.

and valve cam shaft (43) insures a constant time relation between driving force and valve mechanism as effectively as though all three cams were mounted on the same shaft. Figure 6 shows the outlines of the cams and the relations in which they are mounted on the shafts. Figure 7 is a graphic representation of one complete cycle of the pump.

It is now necessary to describe the tubes which are used to modify the character of the pulse which is developed when an organ such as the kidney is perfused. If constancy of output under conditions of varying peripheral resistance is a desideratum, it is necessary that the tension on the spring (24) shall

considerably exceed the minimal tension necessary to cause a downstroke of the piston. The initial pulse wave is consequently inordinately high and is followed by waves of rebound. To correct these and to obtain a pulse which approximates that of the normal circulation, two glass tubes (9 and 10, fig. 1) are connected in series with the rubber tube (8) which forms the outlet valve of the perfusion pump. The first (9) is a T-tube, of 3.5 mm. bore, the horizontal arm of which is 6.5 cm. long: the vertical arm is 28 cm. long and has two stop cocks 9 cm. apart, the lower being 14 cm. from the horizontal arm. The space in the vertical arm acts as an air cushion which takes up the first impact of the force of the downstroke of the pump. The extra stop cock makes the volume of the air cushion, and so the degree of its effect, susceptible of variation during an experiment.

The second glass tube (10) joined to the first (9) is another stop cock tube, the total length of which is 7.5 cm. The hole through the stopper is 1.5 mm. in diameter: a rather deep notch is filed across the stopper at each end of the hole so that on turning the stopper closure of the cock is more gradual than would

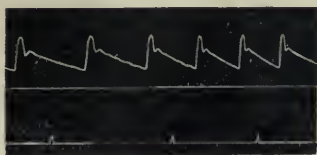


FIG. 8 ($\times \frac{1}{2}$).

otherwise be the case. This cock enables one to further diminish the pulse pressure and to prevent waves of rebound from reaching the vessels of the organ being perfused. We have found it convenient to etch a short scale on the stop cock so that at the beginning of a perfusion it can be set at approximately the position which experience has shown will yield the proper pulse curve. A tracing made with a Hürthle manometer attached to the side arm of the tube (10) is shown in figure 8.

This arrangement enables us to set the spring (24) at a tension so high as to give a vigorous downstroke against any resistance which may be encountered in the course of an experiment and yet to modify character and pressure of the pulse without at the same time altering materially mean pressure or output.

This second stop cock tube is the one which is connected with the artery of the perfused organ: the side arm is connected with

a manometer for recording pressure in the perfused vessels. The bulb of a small thermometer is introduced into the perfusion stream close to the organ by another T-tube.

The cannula in the vein of the perfused organ is attached to a bent glass tube which passes through the rubber stopper of a 130 cc. wide-mouth bottle which forms the intake (venous) reservoir for the oxygenating pump (not shown in drawings). A second glass tube, also passing through the stopper to the bottom of the bottle, is connected with the inlet tube of the oxygenating pump. A third glass tube, straight and of larger diameter than the others (12 mm.) passes through the same stopper and is of use in adding blood to the perfusion system.

The oxygenating device. While we agree with Starling in his dictum that it is impossible to construct an aerating apparatus for blood which shall approximate to the efficiency of the lungs we have made a device which has answered the purpose which the experiments thus far have involved, i.e., the adequate continuous oxygenation of the blood necessary to supply such an organ as the dog's kidney. It has the advantage of not causing any frothing of the blood and its use does not increase the necessary volume of blood in the artificial circulation to an amount greater than can be supplied by the animal (dog) from which the organ is taken.

The apparatus consists of a glass chamber (fig. 9, 56) made by cutting off the bottom and part of the neck of an ordinary square tincture-mouth bottle of one liter capacity. A 1-hole rubber stopper is tightly inserted in the neck. A glass tube (not shown in the drawings) passes through it, the end within the bottle being flush with the surface of the stopper: the longer portion of the glass tube is bent so that, when the chamber is held in the short burette clamp (61) as shown in figure 5, the end of the glass tube meets and can be attached to the rubber tube which forms the inlet tube of the perfusion pump. A

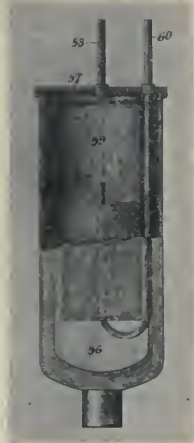


FIG. 9. Oxygenating device ($\times \frac{1}{3}$).

hard rubber cover (57) was made to fit over the mouth of the chamber. Two holes through it allow two glass tubes to pass through and to be fixed in position by rubber collars. The first (58, figs. 9 and 10), 2.5 mm. inside diameter, passes through the center of the cover. It is straight for 6 cm., then bent so that a circle 5 cm. in diameter is formed in a plane at right angles to the straight portion. The lower surface of the entire circular portion is pierced by small holes 0.5 cm. apart.

A rectangular piece of fairly heavy, loosely woven cloth is cut, its width being equal to the circumference of the glass circle and its length about that of the body of the glass chamber. A hem is made along the entire width of the cloth, sufficiently



FIG. 10. Circular glass tube through which blood is driven into the oxygenating device ($\times \frac{1}{3}$).

wide to allow it to be slipped over the circular portion of the glass tube. When this is done the cloth forms a cylinder (59) dependent from the glass circle, the small holes in the glass being covered by the hem of the cloth. When blood is pumped into the straight end of the glass tube it spurts out of the numerous small holes, is caught immediately in the meshes of the cloth and flows slowly in a uniform layer down the cloth.

Another hole in the cover of the chamber admits the second glass tube (60). It is bent in the form of a U, one arm longer than the other, the longer arm being the one fixed in the cover. The length and adjustment is such that when both the tube and the cloth cylinder are in position the edge of the cloth reaches just to the bend of the U and the short arm of the U projects upward in the center of the cloth cylinder. Holes are made in both arms of the U below the cover. Washed oxygen is supplied to the chamber by connecting the end of the U which projects above the cover with an oxygen tank.

The oxygenating chamber is held in position by fixing its neck in a short burette clamp (61) fastened to the round upright (26) which also serves to fasten the lower end of the spring (24). When in position, a suitably bent glass tube connects the outlet

of the oxygenating-pump with the straight end of the circular glass tube. As has been mentioned a glass tube from the bottom of the oxygenating pump leads to the inlet tube of the perfusion pump—the chamber serves as an intake reservoir for the latter. In the passage of the blood over and through the cloth and as it drips from the cloth to the bottom of the chamber no bubbling or frothing occurs.

One practical point in connection with the holes in the circular glass tube is important. We have found it impossible to get the holes small enough and of uniform size. In such a case the blood issues from the tube through only two or three of the holes and the effectiveness of the apparatus is greatly lessened. To overcome this difficulty we have had the holes made purposely a little too large, coated the inside of the tube with paraffin thereby filling up the holes completely and then with a fine needle pricked holes of the proper size through the paraffin.

We have not made exhaustive measurements of the efficiency of this part of the apparatus. In every experiment in which it has been used, however, the arterial hue of the blood which had passed through it was striking in comparison with the venous hue of that issuing from the perfused organ. The cloth first used was heavy, loosely-woven linen. In one test, defibrinated ox blood which had become reduced by standing in a closed vessel was pumped through the apparatus at the approximate rate of 50 cc. per minute three successive times. Estimated by Haldane's ferricyanide method, the oxygen content of the reduced blood was 1.5 per cent. After one passage through the chamber it was 7.5 per cent; after the second passage, 15 per cent; after the third, 19 per cent. During the first and second passages, oxygen was forced through the chamber at the approximate rate of 800 cc. per minute; during the third, at 300 cc. per minute.

This sample of cloth was found to be made of such short fibres as to make it impossible to wash it free of lint, and in experiments on the kidney in which it was used infarcts were produced from this cause. The substitution of a heavy pongee silk gave satisfactory results.

Temperature. To maintain the proper temperature of the blood during its passage through the system, the stand is placed in a water bath made of galvanized iron. Its dimensions are

$10\frac{1}{4} \times 10\frac{1}{4} \times 10$ inches. The glass stopcock tube (10, fig. 1) between the air cushion and the perfused organ, passes through a hole in the side of the bath, a water-tight joint between the glass and the edges of the hole being made by a rubber collar. Similarly the glass tube leading from the venous reservoir to the inlet tube of the oxygenating pump passes through the wall of the bath. All connections being water-tight, the bath is filled with water at 38°C . so that its level reaches to within an inch of the top of the glass cylinders of the pumps. To keep the water warm two tubular incandescent electric bulbs are inserted through openings in the sides of the bath at diagonally opposite lower corners. They are sufficient to maintain the temperature of this volume of water at $38\text{--}41^{\circ}\text{C}$. indefinitely.

Before setting up the apparatus it is important that every part be scrupulously cleaned and rinsed with salt solution. The salt solution must be filtered free of all visible particles. The cloth used in the oxygenating device must be of such a character and washed so thoroughly that no lint or other particles will be given off from it to the blood passing through its meshes or over its surface.

The pumps, oxygenating chamber and air cushion tube can be fastened in the support and all connections made before placing the apparatus in the water bath. The stopcock tube (10) is then attached to the air cushion tube and the tube from the venous reservoir to the inlet of the oxygenating pump. After inserting the electric bulbs through the wall of the bath, the bath is filled with water.

To fill the pumps blood is poured into the venous reservoir through the straight tube. A rubber tube connected with a glass bulb (such as a 25 cc. pipette) is attached to the filling tube of the oxygenating pump. With the inlet valve and the stopcock of the filling tube open, blood is drawn through the pump up to the end of the filling tube. If the screw (23) is loosened, the piston can be worked up and down by hand a few times while the blood is being drawn through the pump in this way and all air bubbles are easily removed. The stopcock on the filling tube is then closed and blood which has been drawn

into the bulb of the glass tube returned to the venous reservoir. When this is done, the screw (23) is made fast with the piston in proper position. Enough blood is then pumped into the oxygenating chamber to thoroughly soak the cloth and to give a supply at the bottom of the chamber which shall be adequate for filling the perfusion pump. This is carried out in the same manner as has just been described for the oxygenating pump. When the pumps and all connecting tubes are filled the apparatus is ready for connection with the vessels of the organ to be perfused.

The cannula inserted into the artery of the organ to be perfused should have a side tube sealed in as close as possible to its neck. A rubber tube from this empties into the filling tube of the venous reservoir. The purpose of this is to guard the organ against the cooling which would result if blood which had stagnated in the connecting tube to the artery while final preparations for perfusion were being made should be driven directly through the organ. With a clip on the artery of the organ and the side tube of the cannula open, blood can be circulated through the entire apparatus, including the tube to and cannula in the artery, until the entire amount of circulating blood shall have taken the maximum temperature to which the bath can raise it. When this is accomplished the clip on the artery is removed at the same time that the side tube is clamped and perfusion is begun.

Estimation of rate of blood flow through the organ. It had been our hope to construct an apparatus which would deliver a constant amount of blood per thrust of pump regardless of changes in peripheral resistance. If this were accomplished it would be possible to calculate the delivery of the pump from known thrust and rate. This has not been accomplished in the apparatus described because of the fact that there is a short stretch of elastic rubber tubing between the ends of the glass outlet and inlet tubes of the pumps and the point at which the rubber tubes are compressed by the valve mechanism. For this reason the output against high is somewhat less than against low resistance. The delivery of the pump can be estimated in quite another

way as follows. A glass scale is etched on the bottle which forms the venous reservoir for the lung pump. To estimate the amount of blood flowing through the organ the oxygenating pump is stopped by loosening its screw (23) and either the time required for a measured increase in the volume of the contents of this reservoir is noted or the increase in volume of contents produced by a counted number of strokes of the pump is measured.

Caution is always to be exercised against allowing the reservoir for the perfusion pump (i.e., the bottom of the oxygenating chamber) to become empty while such an estimate is being made.

The development of the apparatus as described has been slow and the results achieved of little value save as they showed the feasibility of the method, and have indicated the probability of its future value. Our first project was the perfusion of the mammalian central nervous system and our experience has led to the conviction that such an experiment is wholly possible: Difficulties arose, however, which led us to try perfusion of the kidney, in the hope that experience gained with the easier problem would facilitate attack on the more difficult. None of the experiments which we have made are perfect enough to warrant detailed description: suffice it to say that in an experiment of May 10, 1913, the central nervous system of a cat was perfused by the vertebral vessels (the innominate artery and subclavian arteries distal to the vertebrae being tied) with the result that after two hours' perfusion the medullary centres were still active: at the close of this experiment the addition of strychnine to the perfusion blood in more than lethal dose for the whole animal caused convulsions only in the upper third of the body and the addition of neutral red to the perfusing blood caused staining of the central nervous system which was limited to the parts above the level of the seventh spinal nerves.

In an experiment of February 26, 1914, a dog's kidney was perfused with blood from the same animal. A slow elimination of fluid from the ureter occurred which was yellow in color, acid in reaction, gave slight positive reaction for albumin which

was less intense than the reaction yielded by bladder urine from the same dog, did not reduce Fehling's solution, gave Jaffé's test for creatinine and underwent ammoniacal fermentation on standing.

We wish to acknowledge our indebtedness to Prof. E. T. Reichert for the use of the facilities of the machine shop of the Department of Physiology and the services of the technician, Mr. Billings; to Prof. J. E. Sweet for most timely assistance in construction of some of the metal parts; to Dr. W. G. Wood and Dr. O. H. Plant for assistance in experiments; and to Mr. Harris Comer for the experimentation which was necessary before the glass pumps could be successfully made.

URINE FORMATION BY THE PERFUSED KIDNEY: PRELIMINARY EXPERIMENTS ON THE ACTION OF CAFFEINE

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The experiments described in this paper represent an effort to determine whether increase in blood flow through the kidney is an indispensable factor in the production of caffeine diuresis.

Those who conceive stimulation of renal epithelium to be the distinctive feature in the action of caffeine upon the kidney base their conception upon the lack of relation (within limits) between the diuretic action and the height of general arterial blood pressure (von Schröder¹): upon the fact that in caffeine diuresis the osmotic pressure of the urine may sink below that of the blood (Dreser²): that in some instances the oncometer does not reveal an increase (vaso-dilatation) in the volume of the kidney during caffeine diuresis (Gottlieb and Magnus³): and that caffeine increases markedly the gaseous metabolism of the kidney (Barcroft and Straub⁴).

Those who deny the force of this evidence look upon the vascular change in the kidney or interference with reabsorption in the tubules or both as the causes of caffeine diuresis. Failure of caffeine to increase sugar elimination in phlorhizin glycosuria and failure to increase phosphate elimination (Loewi⁵) is taken

¹ von Schröder: Arch. f. exp. Path. u. Pharm., 1887; xxii, p. 39; 1888, xxiv, p. 85.

² Dreser: Ibid., 1892, xxix, p. 303.

³ Gottlieb and Magnus: Ibid., 1901, xlv, p. 223.

⁴ Barcroft and Straub: Journ. of Physiol., 1910-11, xli, p. 145.

⁵ Loewi: Arch. f. exp. Path. u. Pharm., 1902, xlviii, p. 410.

as evidence that secretory processes in the kidney are not stimulated. It is held that dilatation of renal vessels is a constant element of causal importance in caffeine diuresis and those experiments in which the volume of the kidney did not increase during caffeine action are classed with Loewi's⁶ encapsulation experiments in which vasodilatation was proved in conditions which precluded the possibility of increase in kidney volume.

Interference with reabsorption in the tubules was suggested by the experiments of von Sobieranski⁷ and is made to appear more probable by those of Grünwald⁸ which showed the reappearance of chlorides in the urine of chloride-starved rabbits following diuretin, and by those studies⁹ in which the osmotic pressure and sugar content of the medulla of the kidney were found to approach those of the cortex during caffeine diureses.

It may fairly be said that our knowledge of kidney function is insufficient to permit a wholly convincing analysis of the effect of a drug upon it. The various factors in urine formation are so intricate that the personal factor must inevitably play a part in the acceptance of explanations of drug actions.

The experiments here described were designed with the purpose of holding constant one factor of universally admitted importance in urine formation—viz.: blood flow through the kidney—and to determine whether caffeine would then be able to induce diuresis. In the event of a positive result, we should be able to say that while the element of renal vasodilation might be a contributing factor to caffeine diuresis, it is not the essential one: and it would give us surer ground from which to approach the positive side of the problem.

It may be stated here that results obtained have been so decisively positive that we feel justified in the conviction that the increased blood flow through the kidney consequent upon renal vaso-dilatation is not an essential factor in caffeine diuresis.

⁶ Loewi, Fletcher and Henderson: *Ibid.*, 1905, liii, p. 15.

⁷ von Sobieranski: *Ibid.*, xxxv, 1895, p. 144; *Arch. f. d. ges. Physiol.*, 1903, xeviii, p. 135.

⁸ Grünwald: *Arch. f. exp. Path. u. Pharm.*, 1909, lx, p. 360.

⁹ Nishi: *Ibid.*, 1910, lxii, p. 329; Hirokawa: *Hofmeister's Beiträge*, 1908, xi, p. 458.

We are aware of three experiments having a similar purpose. Starling¹⁰ in his study of the effects of hydraemia upon urine formation kept the kidney volume (oncometer) constant during the hydraemia following sugar injection by bleeding from the carotid. He found that diuresis did not occur and concluded from this and other lines of evidence that renal vaso-dilatation induced by hydraemia was the chief renal factor involved. Cushny¹¹ in studying saline (NaCl) diuresis accomplished the same purpose by constricting the renal artery to such an extent that the oncometer record remained stationary. Failure of diuresis to occur convinced him that the cause of saline diuresis was to be found in the vascular change induced.

It is to be noted that these observations suffer from the limitations of all oncometric experiments: and that in the absence of direct measurement there can be no guarantee that the blood flow through the kidney was lessened only to the extent necessary to compensate for the vascular change.

Bainbridge and Evans¹² have recently adapted the Knowlton-Starling heart-lung preparation to perfusion of the kidney and found that sodium sulphate caused diuresis when blood flow through the kidney was constant.

METHODS

The method employed utilized the perfusion apparatus described in the previous paper—modified somewhat in its application. A single pump only was used, the aeration of the blood which was pumped through the kidney taking place in the animal's own lungs. The attachments of the perfusion pump were the same as those described on p. 476 with the exception that the intake tube of the pump was connected with a small reservoir supported at the side of the water bath into which blood from the animal's carotid flowed at the same rate as that at which blood was pumped into the animal's renal artery. The

¹⁰ Starling: *Journ. of Physiol.*, 1899, xxiv, p. 317.

¹¹ Cushny: *Ibid.*, 1902, xxviii, p. 441.

¹² Bainbridge and Evans: *Ibid.*, July, 1914, xlviii, p. 278.

reservoir has a total capacity of 12 cc. It is made of glass tubing constricted at two points: a mark is etched on the glass at each constricted point and the capacity of the space between the marks is 5 cc.

To estimate rate of delivery of the pump to the kidney the stream of blood from the animal's carotid was temporarily diverted to another receptacle and the time required for the level of the blood in the reservoir to sink from the higher to the lower mark was recorded by a signal.

Operation. Rabbits were the animals used. They were fed on carrots during the day preceding the experiments. Anaesthesia was produced by injection of 2.0 gm. of urethane per kilo into the stomach. A little ether was given during the operation. The trachea, both carotids and, in experiments 1 and 2, left external jugular vein were prepared for cannulas. The abdomen was opened by median incision from symphysis to sternum. Coeliac axis, superior and inferior mesenteric arteries were ligated, and the gastro-intestinal tract from oesophagus to rectum excised together with spleen and pancreas. The abdominal aorta and the inferior vena cava were ligated at points about an inch below the renal vessels: a large lumbar artery at that level was ligated. A cannula was tied into the left ureter at a point about an inch above the bladder. The right kidney was excluded from the circulation by a mass ligature about its vessels. A loose ligature was placed about the abdominal aorta at a point between the stumps of the coeliac axis and the superior mesenteric artery. About half an inch below the left renal vessels a cannula was inserted into the abdominal aorta and another into the inferior cava, both pointing toward the heart; both vessels were clamped above the cannulas.

Hirudin (120 mgm. dissolved in 16 cc. of 0.9 per cent NaCl) was slowly injected into the cava through the cannula.

Cannulas were inserted into the right carotid for measurement of the animal's arterial blood pressure: into the left carotid for supplying blood to the perfusion pump: and, in experiments 1 and 2, into the external jugular for injection of substances to be tested.

The entire operation was practically bloodless. Temperature of the animal was maintained by an electric warming pad.

Artificial respiration by a Meyer pump was used in experiment 3, not in experiments 1 and 2.

The assembly of the apparatus is pictured in figure 1.

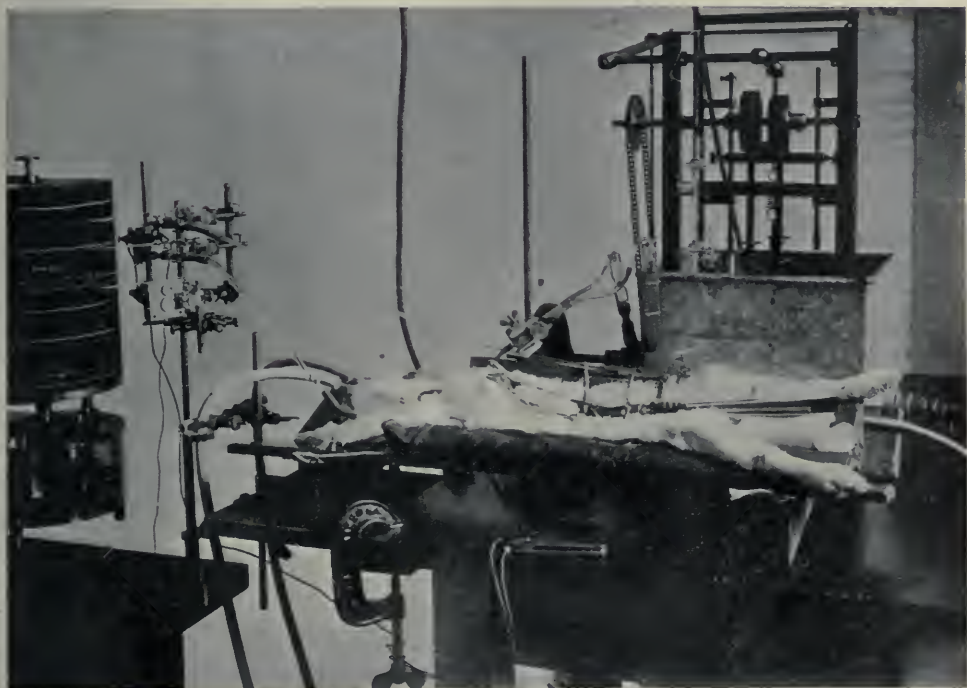


FIG. 1. Photograph of the perfusion and recording apparatus made immediately after the completion of experiment 3.

As soon as the operation was completed, a second rabbit, anaesthetized with urethane at the same time as the first, was bled from the abdominal aorta into a beaker containing 40 mgm. of hirudin in 6 cc. of 0.9 per cent NaCl. Twenty cc. of the blood, the amount necessary to fill pump, reservoir and connections, was used in displacing the salt solution with which the pump and all connecting tubes had been filled before the oper-

ation was begun. The rest was reserved for addition to the perfusion system as the experiment went on.

The vessels of the animal were connected with the perfusion apparatus in the following manner: The cannula in the left carotid was connected by rubber tubing with one limb of a three-way cock bored obliquely so that the blood could be sent through either of two outlets on the other side. One of the outlets emptied into the intake reservoir of the pump; the other into a test tube supported in an improvised wire basket hung on the edge of the water bath. A screw clamp closed the rubber tubing between artery and stop cock.

The delivery tube of the perfusion apparatus was connected with both the inferior vena cava and the abdominal aorta by means of a glass four-way tube, one arm of which permitted the introduction of the bulb of a thermometer into the tube connected with the aorta.

The cannula in the aorta had a side arm close to the neck to which was attached a rubber tube emptying into the intake reservoir of the pump.

In experiment 3 the cannula in the inferior vena cava was provided with a similar side arm through which injections of substances to be tested were made.

When the connections described were made between the perfusion apparatus and the vessels of the animal, all tubes being filled with blood, we proceeded as follows: The tube leading to the aorta was clamped, the clamp on the inferior cava removed and the pump, set at lowest thrust and moderate speed, was started. The blood was thus pumped directly into the cava: to prevent exhaustion of the reservoir blood was allowed to flow into it from the animal's carotid, the screw clamp on the rubber tube being set so that the height of the column of blood in the reservoir remained constant. Under these conditions the animal was bleeding into the pump at the same rate that the pump was driving blood into the animal's veins. The purpose of this was to secure thorough mixing of the blood with which the pump and connections had been filled with that circulating in the animal's body. When this process had continued for

about two minutes, the tube from the carotid was clamped, the tube into the cava clamped and the clamp on the tube to the aorta opened. The blood was then forced by the pump into the aortic cannula, out through its side arm and back to the reservoir of the pump. The purpose of this was to replace the cooled blood which had been standing in the cannula in the aorta and its connecting tube with warmed blood. Then at a given signal the clamp on the aorta was removed, the side arm of the aortic cannula clamped and the loose ligature about the aorta above the renal vessels tied. At the same time the clamp on the tube leading from carotid to reservoir was opened as before.

The circulatory events then taking place were as follows: Blood was withdrawn from the animal's carotid into the reservoir of the pump at the same rate that it was pumped via the aorta into the left renal artery: after passing the kidney it returned to the animal's venous circulation: aeration took place in the animal's lungs.

The time required for these last manipulations was not more than ten seconds: the substitution of the normal circulation through the kidney by the artificial was practically instantaneous: at no time was circulation through the kidney stopped: for ten seconds the organ received blood both from the heart and from the pump.

From then on it was possible to measure as often as desired the amount of blood passing through the pump (and hence through the kidney) and to alter character and volume of blood flow at will.

The implication has been made above that all of the blood pumped into the abdominal aorta of the animal, prepared as described, passed via the renal artery through the kidney. This is not true. Between the cannula and the ligature about the aorta above the renal vessels, the suprarenal arteries and one lumbar artery are given off. We have preferred to leave these patent rather than to injure the nerve supply to the kidney and risk the production of bleeding points, believing that this source of error can hardly be a factor in the results obtained.

Measurement of urine. The cannula inserted into the ureter of the perfused kidney was made from a 2 cc. pipette, graduated in tenths, the neck of the cannula being formed just below the zero line. A small side arm was sealed in at about the same point, by means of which the tube could be emptied. The tube was supported in a nearly horizontal position and urine flow was recorded by noting the position of the meniscus of the column of urine at frequent intervals. Rate of urine flow could not be recorded during the short intervals when the urine tube was being emptied. The gaps in the records (protocols and charts) represent these intervals.

Continuous record was made of the arterial blood pressure in the rabbit's carotid and the pressure in the perfusion tube leading to the aorta by means of Hürthle manometers (Albrecht construction). Frequent records were made of pump delivery, urine flow, and in experiment 3 of temperature of perfusing blood.

Urine analyses. The urine samples obtained during each experiment were combined into portions corresponding to the various phases of the experiment and were subjected to examination as follows: Specific gravity by weighing a small glass bulb, loaded with mercury, while immersed in the urine: freezing point by a Beckmann apparatus with a small tube: total nitrogen and urea by Folin's colorimetric methods: chlorides according to Volhard (1 cc. of urine; $\frac{N}{10}$ AgNO₃; $\frac{N}{20}$ KCNS): creatinine according to Folin (bichromate standard; 1 cc. urine in a 25 cc. flask). In control tests of the methods as applied compared with the same applied to larger quantities of urine, satisfactory agreement of results was obtained.

Reaction was tested by litmus; protein by heat and acetic acid and by picric acid; sugar by Benedict's solution.

The chief purpose of the analyses was to determine whether the composition of the fluid collected from the ureter during perfusion differed materially from that of the fluid collected before perfusion began. We think that this purpose was served: the value of the figures as an analytical picture of events which took place is lessened by the facts that urethane, the anaesthetic chosen, yields all of its nitrogen as ammonia in the Folin estima-

tions and that sodium chloride was used in the hirudin injections and in rinsing the pump. We learned later that hirudin in urine gives both of the tests for protein which were applied.

RESULTS

The results obtained are given in detail in the protocols, Tables I to VI, and are graphically presented in charts 1-3.

1. At the beginning of perfusion urine flow was markedly diminished or ceased entirely (experiment 1, $4\frac{1}{2}$ minutes, 0; experiment 2, 10 minutes, 0.017-0.008; experiment 3, 4 minutes, 0.003-0.022 cc. per minute). In each experiment, however, the initial rate of kidney perfusion was low (22.6, 21.9, 18.4, cc. per minute), and the interruption in urine flow appears to be due to this fact. Promptly after increasing the perfusion rate to 29-30 cc. per minute urine flow was resumed.

2. Rate of urine flow during perfusion at a constant rate is remarkably constant. In experiment 1 during perfusion for 57 minutes, the extremes of rate were 0.1 and 0.33 cc. per minute. During 40 minutes of this time the extremes were 0.175 and 0.31 cc. In experiment 3, during the 22 minutes of perfusion before caffeine was injected, the rate of urine flow varied between 0.105 and 0.196 cc. per minute. Some of these variations are obviously due to the addition of fresh blood to the reservoir necessitated by low arterial pressure of the animal. In experiment 3 we found that the diminution of urine flow which resulted from such addition could be prevented if the blood was slowly infused into the animal's cava instead of being poured into the reservoir and hence we believe that the effect was due to alteration in temperature of the perfusing blood rather than to any specific effect of the fresh blood upon the kidney.

3. There is little evidence in these experiments that a close relationship exists between pressure in the renal circulation and urine flow. Marked changes in pressure occurred, due to changes in calibre of renal vessels, the sympathetic nerve supply to the kidney having been intact. These changes do not, as in the intact animal, cause change in rate of blood flow

TABLE I

Experiment 1. Perfusion of left kidney*

April 10, 1915. Rabbit 2300 gm. Urethane 2.0 gm. per kilo by stomach tube at 8.10 a.m. Operation begun, 9.19 a.m.; finished 10.40 a.m. Weight of kidney, 6 gm.

PERIOD NUMBER	TIME	LEFT KIDNEY URINE		AVERAGE PRESSURE ANIMAL'S CAROTID MM. HG.	PUMP			REMARKS
		cc.	Rate per min- ute cc.		Mean pressure mm. Hg.	Strokes per minute	Delivery cc. per minute	
II	10.36-10.48	1.68	0.14					10.40-10.47 Hirudin-saline injection
	10.50.10-10.54.30	1.4	0.32					
III	10.59-11.09	4.2	0.30					11.09.15 Perfusion started. Thrust 0.†
	11.00.30-11.11	0	0	108	25	120		
	11.11-11.14	0	0	116	40	146	22.6	
IV	11.14.30-11.16.30	0.35	0.175	110	62	166	30.0	11.21. Blood added to reservoir
	11.16.30-11.19.30	0.82	0.270	115	69	160	29.1	
	11.19.30-11.21.30	0.58	0.290	108	64	160	32.6	
	11.21.30-11.24	0.3	0.120	124	60	160	31.2	
	11.24.37-11.26.14	0.5	0.33	116	66	168	33.0	
	11.26.14-11.30	1.13	0.30	104	75	168	30.3	
	11.31.25-11.33.30	0.4	0.20	100	67	164	30.0	
	11.33.30-11.36.35	0.8	0.26	94	82	164	30.3	
	11.36.35-11.39.28	0.8	0.26	97	84	162	30.6	
V	11.40.06-11.43.46	0.9	0.24	96	94	162	30.7	11.40. Urine began to show hemo- globin
	11.43.46-11.46.18	0.6	0.24	90	96	162	30.3	
	11.46.18-11.48.35	0.7	0.31	88	97	162	31.2	
	11.49.36-11.55.40	1.3	0.21	82	105	170	30.2	
	11.55.40-11.59	0.73	0.22	80	119		30.6	
	12.00.25-12.04.25	0.11	0.027	118	78	170	30.0	
	12.04.25-12.07.55	0.36	0.1	120	68	170	30.4	
VI	12.07.55-12.11.27	0.52	0.15	110	81	170	31.2	12.00-12.01. Blood added to reser- voir
	12.12.20-12.15.55	0.89	0.25	90	54	170	33.0	
	12.12.37-12.13.43.							
VII	12.15.55-12.18.25	0.95	0.34	97	60	170	30.3	12.12.37-12.13.43. Caffeine 25 mgm. in 2.5 cc. 0.9 per cent NaCl per ext. jugular. Pump pressure fell from 90 to 50 mm. Hg.
	12.18.55-12.22.20	1.8	0.53	97.5	73	170	31.2	
	12.22.55-12.26.55	1.95	0.49	80	86	170	29.7	
	12.27.20-12.29.45	1.07	0.44	62	98		29.7	
	12.29.45-12.32.10	0.85	0.34	60	100	170	30.0	
	12.32.40-12.35.55	1.05	0.32	54	106	172	29.4	
	12.35.55-12.40.37	0.8	0.17	48	135	172	29.1	

TABLE I—Continued

PERIOD NUMBER	TIME	LEFT KIDNEY URINE		AVERAGE PRESSURE ANIMAL'S CAROTID MM. HG.	PCMP			REMARKS
		cc.	Rate per min- ute cc.		Mean pressure mm. Hg.	Strokes per minute	Delivery cc. per minute	
VIII	12.41.05-12.43.25	0.65	0.28	35	112		30.0	12.46.23-12.48.18, 12 cc. 0.9 per cent NaCl per jugular vein.
	12.43.25-12.47.05	1.1	0.30	36	110	176	32.6	
	12.47.30-12.49.17	1.3	0.72	76	76	180	33.3	
	12.51.30-12.55.25	1.95	0.49	56	96	182	31.2	
IX	12.56.05- 1.00.10	1.3	0.33	43	108	180	30.7	12.59.40-1.01.20, 6 cc. sat. sol. phlorhizin in 0.9 per cent NaCl
	1.00.30- 1.05.12	1.82	0.39	53	99	180	29.85	
	1.05.45- 1.08.25	0.75	0.27	40	112		30.0	
	1.08.25- 1.12.25	1.15	0.28	40	112	180		
	1.12.50- 1.18	1.71	0.33	42	115			

* This experiment was shown in demonstration before the Interurban Clinical Society.

† Thrust of the piston of the pump is expressed in terms of divisions of the scale etched on the driving beam (see p. 473 and figure 4 of the preceding article).

TABLE II

Experiment 1. Urine Analyses

NUMBER	PERIOD	LENGTH OF PERIOD MINUTES	AVERAGE RATE OF SECRETION CC. PER MINUTE	SPECIFIC GRAVITY	FREEZING POINT	MG. PER CC.				MG. PER MINUTE		
						Total nitrogen	Urea nitrogen	Creatinine	Chlorides	Urea nitrogen	Creatinine	Chlorides
I	Bladder urine			1.0179	-0.9	2.94	1.84	0.22	6.17			
II	Before perfusion	12	0.14						7.25			1.01
III	Before perfusion (after hirudin)	18.3	0.30	1.0164	-0.884	2.94	1.54	0.18	6.62	0.47	0.052	1.98
IV	Perfusion	22.8	0.25	1.0169	-0.924	1.68	1.00	0.15	8.08	0.25	0.037	2.02
V	Perfusion	28.7	0.18	1.0156	-0.86	1.73	1.04	0.12	6.8	0.19	0.022	1.22
VI	Perfusion, caf- feine	9.4	0.39	1.0142	-0.802	1.53	1.23	0.13	6.75	0.48	0.048	2.61
VII	Perfusion, caf- feine	16.8	0.34	1.0139	-0.805	1.34	0.98	0.14	6.22	0.33	0.035	2.11
VIII	Perfusion, ad- renalin	5.9	0.3						6.31			1.86
IX	Perfusion, 0.9 per cent NaCl	9.8	0.46	1.0139	-0.775	1.36	0.76	0.11	6.90	0.36	0.052	3.16
X	Perfusion, phlorhizin	16.6	0.33	1.0151	-0.752	1.35	0.77	0.13	5.94	0.24	0.041	1.94

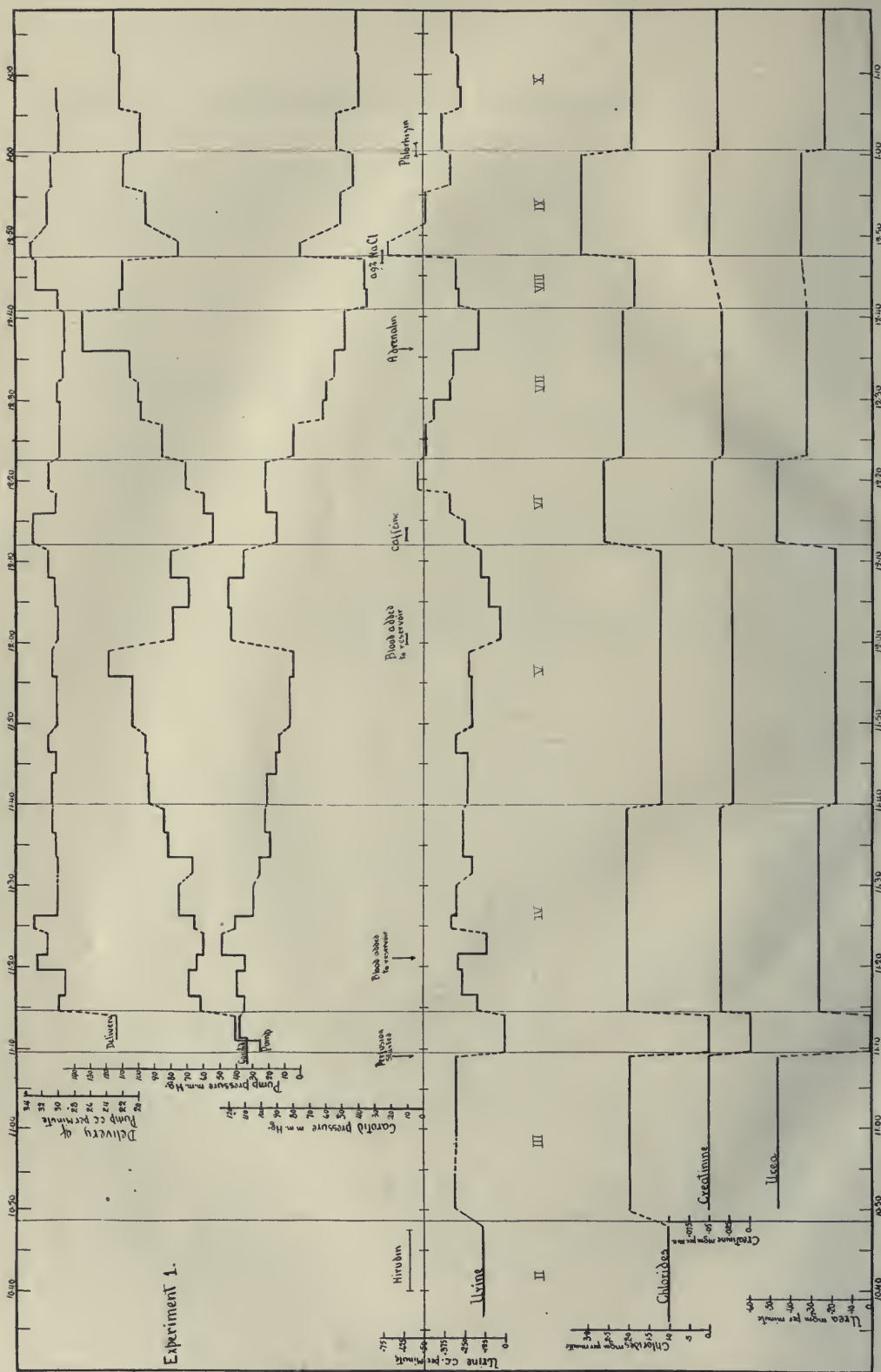


CHART 1.

Experiment 2. Perfusion of left kidney

PERIOD NUMBER	TIME	LEFT KIDNEY URINE		AVERAGE PRESSURE ANIMAL'S CAROTID MM. Hg.	PUMP			REMARKS
		cc.	Rate per min- ute cc.		Mean pressure mm. Hg.	Strokes per min- ute	Delivery cc. per minute	
II	10.34-10.41	0.35	0.05					10.41.30-10.48. Hirudin-saline in- jection
	10.41-10.58	1.75	0.103					
III	10.58.30-11.03.30	2.1	0.42					11.09. Perfusion begun. Thrust 5 11.13. Thrust 7 11.14.45. Thrust 10
	11.03.50-11.07.35	2.0	0.53	86.4				
	11.09-11.13	0.07	0.017	90	40	136	21.9	
	11.13-11.14.45	0.02	0.011	90	42	142	23.4	
IV	11.14.45-11.19.50	0.04	0.008	83.5	60	148	31.1	11.33.05-11.33.30, caffeine 20 mgm. in 1 cc. 0.09 per cent NaCl per jugular vein. Carotid pressure fell to 50 mm. Hg. Pump pres- sure fell to 50 mm. Hg.
	11.19.50-11.23.40	0.25	0.062	86	67	148	31.0	
	11.23.40-11.29.15	0.75	0.136	87	71		30.6	
	11.29.15-11.31.40	0.31	0.124	80	74		29.3	
	11.32.05-11.40.28	2.0	0.24	79.5	70	146	30.1	
V								11.52. Urine began to show hemo- globin. 11.54.10-11.54.40, 10 cc. blood added to reservoir
	11.40.53-11.46.45	1.9	0.32	81	103		28.9	
	11.47.15-11.52.15	1.65	0.33	59	116	144	28.9	
VI	11.52.50-11.54.40	0.6	0.30	47.5	120	144	28.1	12.08.30-12.09.30, 6 cc.. 0.9 per cent NaCl per jugular vein 12.17-12.18.10, 10 cc. 0.9 per cent NaCl per jugular vein 12.30.50-12.31.10, 10 cc. blood added to reservoir 12.47-12.47.30, 2 cc. 5 per cent NaCl per jugular vein
	11.54.40-12.00.10	0.43	0.08	93	70		30.6	
	12.00.10-12.05.50	0.97	0.17	78.5	85	146	30.0	
	12.06.34-12.13.48	2.0	0.27	70.8	83	146	30.15	
	12.14.10-12.21	2.0	0.29	70	74	144	29.7	
	12.21.20-12.29.25	2.0	0.25	50	75		29.1	
	12.30-12.44	0.4	0.028	73	56	146	29.3	
VII	12.44-12.49	0.22	0.044	58.4	77.5	142	28.5	1.02.40-1.04, 10 cc. blood per inf. cava. 1.03.42-1.07.30 1.07.30-1.10.07 1.10.07-1.14 1.14-1.16
	12.49-12.52.30	0.38	0.108	63	76.5	146	29.4	
	12.52.30-12.56.15	0.6	0.16	53	79	144	28.6	
	12.56.15-12.58	0.3	0.17	44	80			
	12.58.27-1.03.42	0.7	0.133	38	80	143	27.8	
	1.03.42-1.07.30	0.4	0.107	63	71		29.4	
	1.07.30-1.10.07	0.3	0.113	57	74		29.7	
	1.10.07-1.14	0.24	0.06	56	65			
	1.14-1.16	0.16	0.08	47	81	144	29.1	

TABLE IV
Experiment 2. Urine analyses

NUMBER	PERIOD	LENGTH OF PERIOD MINUTES	AVERAGE RATE OF SECRETION CC. PER MINUTE	SPECIFIC GRAVITY	FREEZING POINT	MGM. PER CC.				MGM. PER MINUTE		
						Total nitrogen	Urea nitrogen	Creatinine	Chlorides	Urea nitrogen	Creatinine	Chlorides
I	Bladder urine			1.0134	-0.491	1.74	1.25	0.41	3.23			
II	Before perfusion											
	(hirudin inj.)	24	0.089					0.53	4.93		0.046	0.44
III	Before perfusion	8.75	0.468	1.0114	-0.818	1.38	0.95	0.20	7.09	0.44	0.092	3.32
IV	Perfusion	23	0.062					0.27	6.90		0.016	0.43
V	Perfusion, caf- feine	19.3	0.287	1.0136	-0.888	1.41	0.92	0.18	6.62	0.26	0.051	1.90
VI	Perfusion, caf- feine	13.16	0.15					0.23	6.68		0.034	1.0
VII	Perfusion, 0.9 per cent NaCl	22	0.27	1.0099	-0.835	1.33	0.81	0.20	6.55	0.22	0.052	1.8

through the kidney because of the excess of power of the pump and absence of compensating vascular channels. Only in exceptional instances is there coincidence between a rise of pressure in renal circulation and increase in urine flow: in quite as many instances is the relation reversed.

On the other hand a close relation between rate of blood flow through the kidney and urine flow is seen. This has been pointed out in connection with the interruption of urine flow at the beginning of perfusion. It is especially obvious in experiment 3, caffeine period, when reduction of perfusion from 25.2 cc. to 19.6 cc. per minute caused the urine flow to decrease from 0.63 to 0.30 cc. per minute.

4. Analysis of the urines obtained during perfusion before caffeine gives no basis for denying that the fluid obtained during perfusion is urine or that the processes concerned in its elaboration differ from those similarly concerned before perfusion. It would be useless to attempt a detailed interpretation of the analytical results—if for no other reason, because of the disturbing factors in the analyses which have been mentioned. While the claim could hardly be maintained that in any period of the experiment the kidney is secreting "normal" urine, because of the anaesthetic, operation, hirudin, etc., neither can it be

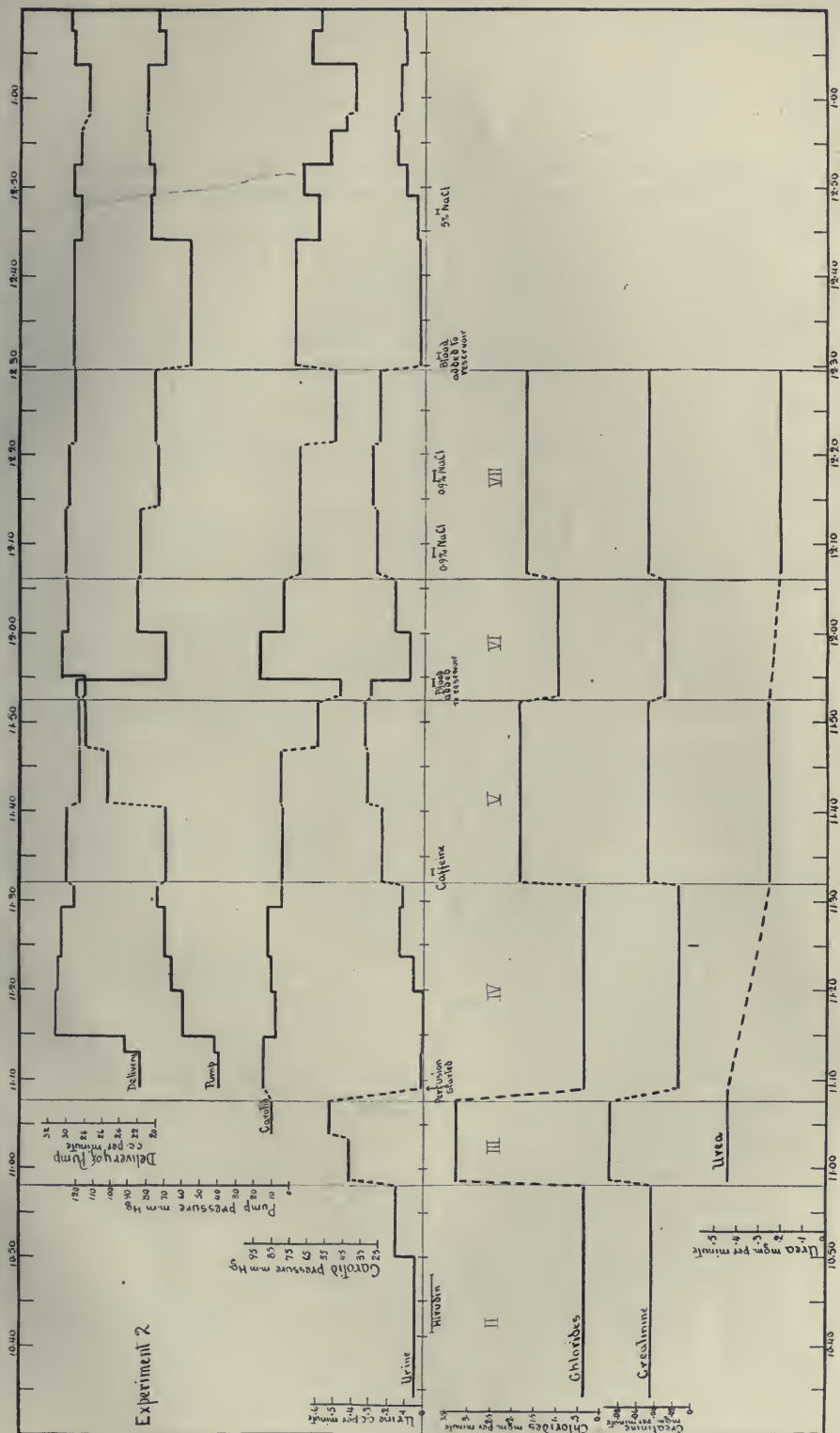


CHART 2.

TABLE V

Experiment 3. Perfusion of left kidney

June 30, 1915. Rabbit 2200 gm. Urethane 2.0 gm. per kilo by stomach tube at 9.40 a.m. Operation begun at 10.20 a.m: finished, 11.35 a.m. Weight of kidney 8 gm.

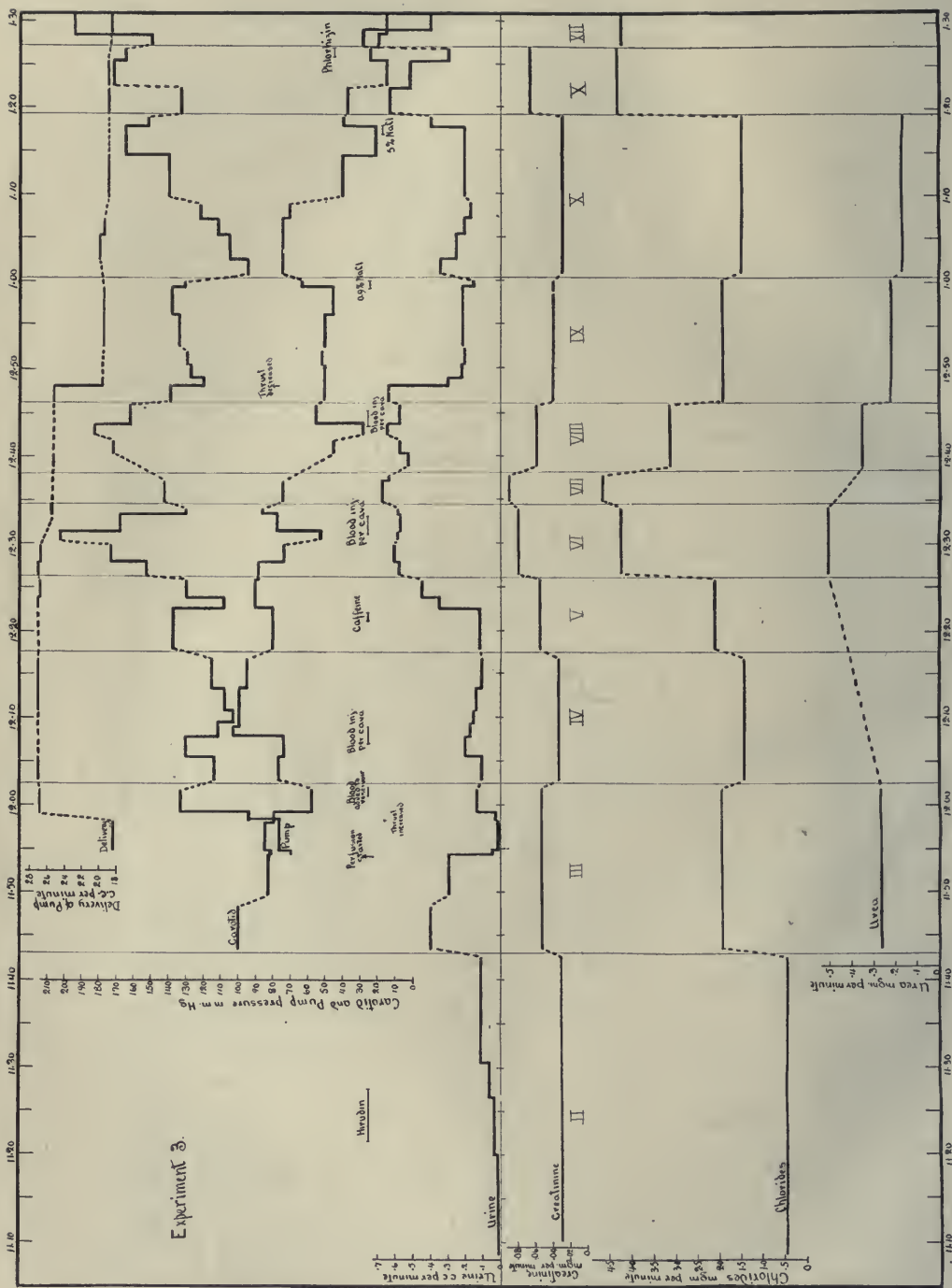
PERIOD NUMBER	TIME	LEFT KIDNEY URINE		AVERAGE PRESSURE ANIMAL'S CAROTID MM. Hg.	PUMP			REMARKS
		cc.	Rate per minute cc.		Mean pressure	Strokes per minute	Delivery cc. per minute	
II	11.08.30-11.20	0.16	0.014					11.21.30-11.27.30. Hirudin-saline injection
	11.20-11.26.30	0.19	0.029					
	11.26.30-11.30.30	0.27	0.068					
	11.30.30-11.42.30	1.41	0.117					
	11.43.23-11.48.18	2.0	0.41	100				
III	11.49.34-11.54.18	1.39	0.29	82.5				11.54.10. Perfusion begun. Thrust 0
	11.54.18-11.54.55	0.03	0.049	82	70			
	11.54.55-11.58.10	0.01	0.003	88.7	76.6	156	18.4	
	11.58.10-11.59.05	0.02	0.022	79	94			
	11.59.05-12.01.48	0.38	0.134	57	133	156	26.8	
IV	12.02.38-12.05.29	0.30	0.105	76.5	113	158	27.0	12.01-12.02. 7 cc. blood added slowly to reservoir 12.07-12.08.50. 9 cc. blood inj. per inf. cava
	12.05.29-12.07.47	0.45	0.196	74.6	130			
	12.07.47-12.09.15	0.25	0.17	103	111.6			
	12.09.15-12.10.43	0.23	0.156	100	102			
	12.10.43-12.13.24	0.37	0.14	100	107	158	27.0	
V	12.13.24-12.16.47	0.36	0.11	95.5	115	158	27.0	12.21.10-12.22. Caffeine, 20 mgm. in 1 cc. 0.9 per cent NaCl inj. per inf. cava
	12.17.47-12.22.34	0.58	0.12	81.6	137			
	12.22.34-12.23.54	0.47	0.35	90	108	160	27.0	
	12.23.54-12.25.49	0.9	0.45	90	129	162	26.8	
	12.26.28-12.27.52	0.8	0.57	88	152.6	164	27.0	
VI	12.27.52-12.29.51	1.2	0.60	74	173	162	26.6	12.31-12.33, 9 cc. blood inj. per inf. cava
	12.30.22-12.31.17	0.5	0.556	53	203			
	12.31.17-12.33.16	1.1	0.55	77.5	168			
	12.33.16-12.33.58	0.4	0.57	86.5	130	158	25.4	
	12.34.42-12.37.05	1.6	0.67	75	142.5			
VII	12.37.05-12.37.43	0.4	0.63					12.38 Urine began to show hemo- globin
	12.38.40-12.40.13	0.8	0.52			152	25.4	
	12.40.13-12.41.58	1.0	0.57	45	172			
	12.42.30-12.43.46	0.8	0.64	27.5	182.5			
	12.43.46-12.45.54	1.2	0.56	56.2	162			
VIII	12.46.16-12.48	1.1	0.63	50	139	154	25.2	12.48. Thrust decreased to 1
	12.48-12.49	0.3	0.3	50	120	154	19.6	
	12.49-12.50.22	0.3	0.22	50	127			
	12.50.22-12.51.48	0.3	0.21	52	129			
	12.52.20-12.56	0.8	0.22	50	134	154	19.5	
IX	12.56-12.59.14	0.7	0.22	46	138	154	19.5	12.59.05-12.59.55, 10 cc. 0.9 per cent NaCl per inf. cava
	12.59.14-1.00	0.12	0.16	64	131			

TABLE V—Continued

PERIOD NUMBER	TIME	LEFT KIDNEY URINE		AVERAGE PRESSURE ANIMAL'S CAROTID MM. Hg.	PUMP			REMARKS
		cc.	ate per minute cc.		Mean pressure	Strokes per minute	Delivery cc. per minute	
X	1.00.45-1.02.30	0.6	0.34	75	95			1.16.50-1.17.15, 2.0 cc. 5 per cent NaCl per inf. cava
	1.02.30-1.05.14	0.7	0.25	75	105	154	20.0	
	1.05.14-1.07.07	0.4	0.21	75	112	152	19.5	
	1.07.07-1.08.50	0.3	0.17	66	122			
	1.09.39-1.14.25	1.0	0.21	40	147	154	19.0	
	1.14.25-1.17.46	0.7	0.21	22.5	165			
XI	1.17.46-1.18.48	0.4	0.39	40.	152			1.25.45-1.26.45, 9 cc. sat. sol. phlor- hizin in 0.9 per cent NaCl, per inf. cava
	1.19.10-1.22.08	1.9	0.64	37.5	133	154	19.0	
	1.22.28-1.25.20	1.5	0.52	15	175	154	19.0	
	1.25.20-1.26.40	0.4	0.30	25	165			
XII	1.27.10-1.28.35	1.0	0.78	20	150			
	1.28.35-1.30.38	0.8	0.39	15	195	152	18.7	

TABLE VI
Experiment 3. Urine analyses

NUMBER	PERIOD	LENGTH OF PERIOD MINUTES	AVERAGE RATE OF SECRETION CC. PER MINUTE	SPECIFIC GRAVITY	FREEZING POINT	MG. PER CC.				MG. PER MINUTE		
						Total nitrogen	Urea nitrogen	Creatinine	Chlorides	Urea nitrogen	Creatinine	Chlorides
I	Bladder urine			1.0055	-0.458	1.02	0.97	0.15	1.50			
II	Before pertusion (hirudin)	34	0.063					0.48	7.59		0.030	0.48
III	Before perfusion	17.11	0.223	1.0114	-0.958	1.58	1.18	0.23	8.72	0.26	0.052	1.95
IV	Perfusion	14.15	0.138					0.24	10.47		0.033	1.45
V	Pertusion (part caffeine)	7.7	0.253					0.22	8.28		0.055	2.09
VI	Perfusion, caffe- ine	7.	0.571	1.0104	-0.784	1.12	0.90	0.14	7.4	0.52	0.080	4.23
VII	Perfusion, caffe- ine	3	0.662					0.14	7.04		0.090	4.66
VIII	Pertusion, caffe- ine	6.7	0.447	1.0117	-0.722	1.31	0.78	0.13	6.97	0.35	0.059	3.12
IX	Perfusion, caffe- ine (decreased thrust)	13.15	0.275	1.0129	-0.72	1.82	0.78	0.15	7.12	0.21	0.040	1.96
X	Perfusion, 0.9 per cent NaCl	17.2	0.232	1.0124	-0.702	1.79	0.72	0.13	6.86	0.17	0.030	1.59
XI	Perfusion, 5 per cent NaCl	7.16	0.53	1.0112	-0.705	1.36	0.58	0.13	8.14		0.066	4.31
XII	Perfusion, phlorbizin	3.46	0.548						7.70			4.22



claimed that the normal processes of urine formation are in abeyance nor that any abrupt change from the operation of normal to abnormal processes occurs when perfusion is inaugurated. Comparison of the figures for chlorides shows that the rate of chloride elimination follows rate of water elimination alike before and during perfusion: percentage variations are to be expected, but in experiment 1 in the second period after perfusion began both rate of output and percentage are closely similar to those in two periods before perfusion. Figures for creatinine vary throughout in the same sense as do those for urine volume. The general course of the percentage curve shows a downward trend, no greater during perfusion than before.

Albumin tests on urine collected before and during perfusion do not give definite information because of the fact that hirudin added to urine reacts positively to both. It can be said that the intensity of the reactions was no greater during perfusion than before, except in samples which contained haemoglobin or blood.

It will be noted that in each experiment haemoglobin appeared in the urine: in experiment 1, 21 minutes after perfusion was started; in experiment 2, 38 minutes, in experiment 3, 43 minutes. Examination of the blood plasma at the close of each experiment showed the presence of free haemoglobin in greater concentration than in any urine sample. Liberation of haemoglobin from corpuscles occurs in the passage of blood through the pump, accomplished chiefly, as experiments to test this point have shown, by the mechanical injury to which they are subjected in the closure of the valves of the pump. It seems clear that the presence of haemoglobin in the urine in these experiments is not indicative of renal abnormality.

In experiment 3 traces of whole blood appeared in the urine, but it appeared before perfusion began.

The only samples of urine which reacted positively to Benedict's solution were those collected after injection of phlorhizin at the close of experiments 1 and 3. It is universally held that phlorhizin produces glycosuria by action on the kidney and injections of this substance have been used as tests of kidney function. The fact that phlorhizin almost instantly gave glyco-

suria in these experiments must be interpreted as indicating that even after long continued perfusion the normality of the renal processes concerned in this phenomenon is not destroyed.

The criticism which all perfusion experiments most frequently meet is that conditions are established which are so far removed from those obtaining in the intact animal that conclusions drawn from them cannot be applied to problems concerned with the intact animal—at least without the greatest caution. Without denying the validity of the criticism in many instances, it is evident that the technique in these experiments has been adequate to permit the retention by the kidney of its power to form urine. And when we show that caffeine in these experiments exhibits the power to produce marked diuresis it cannot be claimed that we are dealing with a process or processes which are not concerned in caffeine diuresis in the intact animal.

5. The injection of caffeine into the animal is followed by diuresis. This result was obtained in each of the three experiments and also in two others not described in this paper because of faulty technique.

In experiments 1 and 3 the increase in urine flow began in less than one minute after the end of the injection: in experiment 2, in three minutes.

The maximum rate of urine flow attained in experiment 1 amounted to $3\frac{1}{2}$ times the rate immediately before caffeine was given (0.15–0.53), a figure nearly twice as great as any previous rate. In experiment 2, the maximum attained was $2\frac{4}{5}$ times that of the rate preceding injection (0.124–0.330) but not as high as during the diuresis produced by the hirudin-NaCl injection before perfusion. In experiment 3, the maximum rate obtained was $5\frac{1}{2}$ times that determined before injection (0.12–0.67), almost $3\frac{1}{2}$ times greater than in any previous perfusion period (0.196–0.67): and more than $\frac{1}{2}$ greater than during diuresis from hirudin-NaCl before perfusion. The extent of the increase and the rapidity with which it is established make it impossible to attribute it to chance variation independent of the caffeine injection.

Duration of diuresis amounted to about 20 minutes in experiment 1: 20 minutes in experiment 2, and 26 minutes in experi-

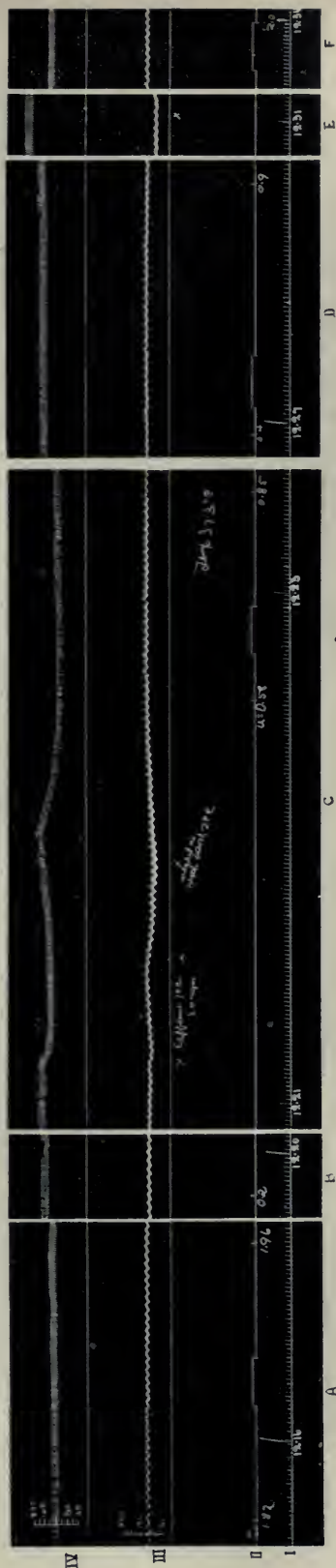


Fig. 2. Portions of record made during experiment 3. Curve I records time in seconds; II is signal for recording measurements of urine flow and blood flow through the kidney. The figures under the line represent positions of meniscus of urine column in the 2-cc. urinary cannula. The time represented by the raised parts of the line is that required in the delivery by the pump of 5 cc. of blood to the kidney. III is the curve of animal's blood pressure, measured from the right carotid artery by a Hürthle manometer; IV is the curve of pressure in the perfusion system measured from a side arm of the tube connecting the perfusion pump with the aortic cannula (see 10 in fig. 1 of the preceding paper).

A and B are sections of the tracing before caffeine was given. C and D show the effects of an intravenous injection of 20 mgm. of caffeine dissolved in 1 cc. of water and washed into the vein with 2 cc. of blood. E and F show the effects of injecting 9 cc. of blood into the animal's vein: the injection was made at 12.31-12.33.

ment 3. It seems certain that in experiment 3 it would have continued longer had the perfusion rate not been diminished at the end of this time.

Figures for rate of perfusion show only slight variations during the caffeine period and most of these are in the direction of a decrease rather than an increase. In experiment 3 in which diuresis was most pronounced perfusion rate was remarkably constant during the first half of the caffeine period: for some unknown reason it diminished somewhat during the second half of that period. The abrupt cessation of diuresis when perfusion rate was considerably lessened in experiment 3 is of interest.

In each of these three experiments the pressure in the perfusion system fell after caffeine was injected (see fig. 2, C). This result has invariably been obtained in every experiment in which we have injected caffeine, whether the action on the animal's circulation caused a rise or fall in its arterial pressure. The action is transitory and in these experiments its intensity and duration appear to bear no relation to the intensity and duration of diuresis. It is, of course, obvious that renal vasodilatation in these experiments does not increase the flow of blood through the kidney as would be the case in the intact animal.

The element of increased blood flow being lacking, the caffeine diuresis here obtained must differ from that obtained in the intact animal to a degree and in a sense which depends upon the importance of this factor—an importance which we believe can be estimated in future experiments.

The result has shown that the dilatation of renal vessels caused by caffeine and consequently the increase in rate and volume of blood flow which occurs in the intact animal is not an indispensable factor in the production of caffeine diuresis.

6. In each experiment the effect of injection of 0.9 per cent sodium chloride has been tested and in two experiments that of 5 per cent sodium chloride. These trials are to be regarded as preliminary. In experiment 1 only did any marked diuresis occur from the isotonic solution, the effect in experiments 2 and 3 being too slight to deserve consideration. In experiment 1, furthermore, the rate of perfusion during the sodium chloride

injection was increased. Since we have as yet no quantitative data concerning the effect of increased rate of perfusion on urine flow the experiments must be considered indecisive. Injection of the stronger solution in both instances (experiments 2 and 3) in which it was tried gave diuresis. In neither case was the result complicated by increase of perfusion rate and tentatively it may be said that 5 per cent NaCl, like caffeine, is capable of causing diuresis when rate of perfusion of the kidney is constant.

7. Study of the pressure curves from the animal's carotid and the perfusion system is of interest. In general the two curves are strikingly reciprocal. The trend of the animal's pressure is downward, that of the perfusion system upward. Whenever the animal's pressure is increased as by the addition of blood or salt solution to the reservoir or by injection into the vein, the perfusion pressure promptly falls (see fig. 2, E and F). As yet no experiment has been made in which the sympathetic fibres to the perfused kidney have been cut: no other explanation presents itself than that the pressure changes in the perfusion system represent vascular changes in the kidney which result from changes in the animal's circulation through the vaso-constrictor centre. We should expect them to be absent in an experiment in which the nerves were cut. The fact of present importance has already been emphasized—viz.: that marked changes in perfusion pressure may occur within the kidney owing to alterations in the calibre of its vessels, without the production of corresponding changes in the volume of urine formed, provided perfusion rate is constant. We shall attempt in future experiments to get more specific data concerning the relation of urine formation to pressure changes in the renal circulation on the one hand, and to changes in volume of blood flow through the kidney on the other. In these present experiments, alteration in calibre of kidney vessels is expressed entirely as change in pressure, alteration in volume of perfusion being prevented by the excess power of the pump and the practical absence of vascular paths other than the vessels of the kidney. In the intact animal, changes in the calibre of renal vessels is expressed as change in volume of perfusion: it seems certain that with the technique

here described an experiment can be performed in which compensation for changes in renal pressure can be made by changing the output of the pump so that pressure remains constant. We shall then be able to express alterations in calibre of renal vessels in terms of volume of blood flow and may arrive at an interpretation of the influence of such changes in calibre upon urine formation. Such experiments we propose to undertake in the near future.

The cause of the gradual decrease in the arterial pressure of the animal is not clear. Certainly our explanation of the change in pressure in the perfusion system precludes the acceptance of an explanation based upon depression of vaso-constrictor centre. The reaction of the animal's circulation to an addition of blood or salt solution to its veins leads us to think that the change is due to or is the equivalent of actual loss of blood from the vessels. There was very slight oozing of blood from dissected surfaces as a result of the hirudin. This alone would not explain the change for in some experiments its amount was negligible. When the pressure in the renal vessels was high there was slight loss of blood around the piston of the pump: this alone is insufficient to explain the change. It must be remembered that we have ligated the abdominal aorta and inferior cava below the level of the renal vessels: it may be that there is a slow accumulation of blood in the capillaries and veins of the occluded area, reaching it by anastomosing arteries. There may be an increase in the capacity of the non-occluded venous reservoirs of the animal with decrease in venous pressure to such an extent as to lessen the amount of blood entering the right heart. There is nothing in the experiment to indicate that primary cardiac failure is the cause.

SUMMARY

1. A method is described for perfusing the rabbit's kidney *in situ* with hirudinized blood under conditions which permit adjustment and measurement of blood flow through the kidney.
2. When blood flow through the perfused kidney amounts

to 28-30 cc. per minute, fluid is eliminated from the ureter which is comparable in composition with that secreted before perfusion was begun.

3. Rate of urine secretion by the perfused kidney is relatively constant when blood flow through the kidney is constant: it appears to be independent of changes of pressure in the renal circulation, provided (as in these experiments) changes in pressure do not cause changes in volume of blood flowing through it.

4. Caffeine causes diuresis in the perfused kidney when the rate of blood flow through the kidney remains constant. The increase in blood flow which occurs in the intact animal as the result of the action of caffeine upon the renal vessels is therefore not an essential factor in caffeine diuresis.

5. It is questionable whether 0.9 per cent sodium chloride (5-6 cc. per kilo) is capable of causing diuresis when blood flow through the kidney is constant: the same amount of sodium chloride, given as 5 per cent solution, is diuretic, even when the rate of kidney perfusion does not change.

6. Phlorhizin causes both diuresis and glucosuria in the perfused kidney.

THE ACTION OF PHENYLETHYLAMIN ON THE HEART¹

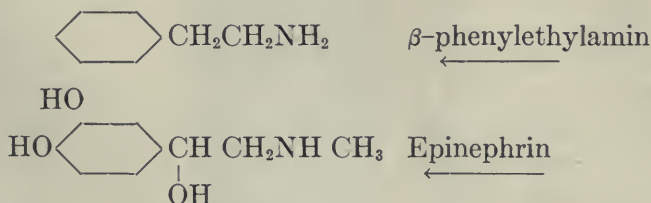
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Phenylethylamin possesses a twofold importance, its derivation from phenylalanin and its physiological potency. Barger and Walpole² recognized it as a pressor constituent of putrid meat, and it may be formed from phenylalanin by the splitting off of CO₂. A similar change is known to take place in the intestinal tract when p. oxyphenylethylamin is formed by bacterial action from tyrosin.

Secondly, phenylethylamin is possessed of many pharmacological actions similar to those of epinephrin, so that Barger and Dale³ refer to it as a "sympathomimetic amine." Its structure in fact is not unrelated to that of epinephrin, the carbon-skeleton being identical:



Phenylethylamin and some closely related compounds are being investigated by one of us (B.) in relation to uterine and

¹ The expenses of this research were defrayed in part from a grant from the committee on therapeutic research, Council of Pharmacy and Chemistry, American Medical Association, and in part from the Francis E. Loomis Research Fund of the Yale University School of Medicine.

² Barger, G. and Walpole G. S.: *Journ. of Physiol.*, 1909, 38, 343.

³ Barger, G. and Dale, H. H.: *Journ. of Physiol.*, 1910, 41, 19.

circulatory activity in decerebrate cats. In the course of this work the impression was gained that the blood pressure changes from this substance were largely cardiac in origin. A closer investigation of the action of phenylethylamin upon the heart was therefore undertaken.

We have performed three series of experiments, showing its action (1) when perfused through the frog's heart, (2) on the surviving isolated heart of the rabbit, and (3) on the heart of the living cat.

β -phenylethylamin hydrochlorid, prepared and kindly furnished to us by Prof. Treat B. Johnson, was used throughout this work.

(1) ACTION ON THE FROG'S HEART

Method. The hearts of pithed frogs were perfused in the usual manner from two Mariotte bottles in which equal and constant pressures were maintained. The inflow cannula was placed in the inferior vena cava, the outflow being through the opened carotids. The perfusion fluid employed was A. J. Clark's⁴ modification of Ringer solution for the frog heart (NaCl 0.7 per cent, KCl 0.014 per cent, CaCl_2 0.012 per cent and NaHCO_3 0.02 per cent). To portions of this solution phenylethylamin-hydrochlorid was added to make concentrations varying from 1 : 50,000 to 1 : 500.

Results. It was found that concentrations of 1 : 50,000 and 1 : 25,000 had no effect upon rate or amplitude although perfused continuously for half an hour. 1 : 15,000 phenylethylamin was the minimal effective concentration, producing within a few minutes some diminution in the amplitude, the rate being unchanged.

Phenylethylamin solutions of from 1 : 10,000 to 1 : 500 concentration gave constant results: diminution in rate and amplitude of the heart-beat. This was seen in nineteen experiments of which figure 1 is a typical example. Reperfusion with Clark's solution restored the heart, which often regained its original rate and amplitude even after the strongest concentrations of

⁴ Clark, A. J.: J. Pharm. and Exp. Therap., 1913, iv, 403.



FIG. 1. (All tracings read from left to right.) Action of frog heart perfused with phenylethylamin hydrochlorid. (Concentration 1:5,000.) Recovery with Clark's solution. Time given in 5 second intervals.

phenylethylamin. In the only case in which acceleration of the rate was observed it was brief and was preceded and followed by slowing. This result was repeated in the same heart after recovery but could not be obtained in other hearts, using the same concentration (1 : 500).

Phenylethylamin therefore in concentration of from 1 : 10,000 to 1 : 500 affects the frog heart by diminishing both rate and amplitude. The action is reversible.

(2) ACTION ON ISOLATED RABBIT HEARTS

A further analysis of the cardiac action of phenylethylamin hydrochlorid was made from a series of experiments upon the surviving hearts of full-grown rabbits.

Method. The animals were decapitated and the blood collected into a vessel containing hirudin dissolved in Locke's solution (NaCl 0.89 per cent; CaCl_2 , 0.024 per cent; KCl, 0.042 per cent; NaHCO_3 , 0.02 per cent). The blood mixture was filtered and further diluted with one to two parts of Locke's solution.

The heart, after prompt excision and cleansing, was connected with a modified Locke and Rosenheim⁵ perfusion system. From a thread connected with the recording lever the heart was suspended apex upward. In this way there was no leakage back into the right heart of the venous blood which flowed directly into the tipper recorder, giving a uniform record of coronary flow under normal conditions. To insure a thorough washing out of the coronary system the heart was first perfused with oxygenated Locke's solution alone, and after several minutes the oxygenated blood mixture was substituted.

Phenylethylamin hydrochlorid was injected obliquely through the rubber tubing a few centimeters from the point of entry of the perfusing fluid into the heart. Changes in arterial pressure were excluded by injecting slowly and with the point of the needle directed against the current. In nearly every case the phenylethylamin was given in 1 cc. of Locke's solution.

⁵ Locke, F. S. and Rosenheim, O.: Journ. of Physiol., 1907-1908, xxxvi, 205.

Results. The change from Locke's solution to the blood mixture causes a marked improvement in the amplitude of the ventricular contraction although always associated with a constriction of the coronary vessels, as indicated by the diminution in coronary flow (fig. 2).

If a small dose, say $\frac{1}{20}$ mgm., of phenylethylamin be injected in the above described manner into the heart of a rabbit a characteristic response is obtained. The amplitude of the ventricular stroke becomes greater in both systole and diastole until (as in fig. 3) each excursion of the recording lever may be increased by one-half its original length. This occurs without definite alteration in the frequency of the beat. There is however another very marked change, the diminution of the coronary flow. The vessels become markedly constricted, to such an extent in fact that the flow may be diminished by half, as in the experiment cited (fig. 3). Thus we find the heart performing, for a short time, a greatly increased amount of work with a much diminished supply of nutrient material. The effect of the drug passes off in three or four minutes. Similar effects have been obtained with a dose as small as $\frac{1}{30}$ mgm. of phenylethylamin showing that the heart is quite sensitive to this substance.

The typical effect of phenylethylamin in doses of 1 mgm. is very different from that produced by $\frac{1}{20}$ of this amount. Here one sees constantly a slowing of the rate and a diminution in the amplitude, as in the frog. As the dose is increased above this amount the effects become more pronounced. They are associated with marked decrease in coronary flow, the constant primary effect of the injection of phenylethylamin in all adequate doses.⁶

But there is a secondary series of effects, of rather an unusual nature, which follows the injection of 1 or more mgm. in the isolated heart. After three or four minutes of depressed activity the heart begins to take on new life not only regaining its former amplitude and rate but sometimes attaining a doubled or trebled excursion and speed. In other words it may perform perhaps

⁶ Phenylethylamin also constricts the coronary vessels of the isolated monkey heart. (Unpublished observations of Barbour and Prince.)



FIG. 2. Isolated rabbit's heart perfused with autogenous hirudinized blood diluted with Locke's solution. 1. Ventricular amplitude. 2. Coronary flow. 3. Time in 5 second intervals. Change from Locke's solution to the blood mixture is shown by arrow.

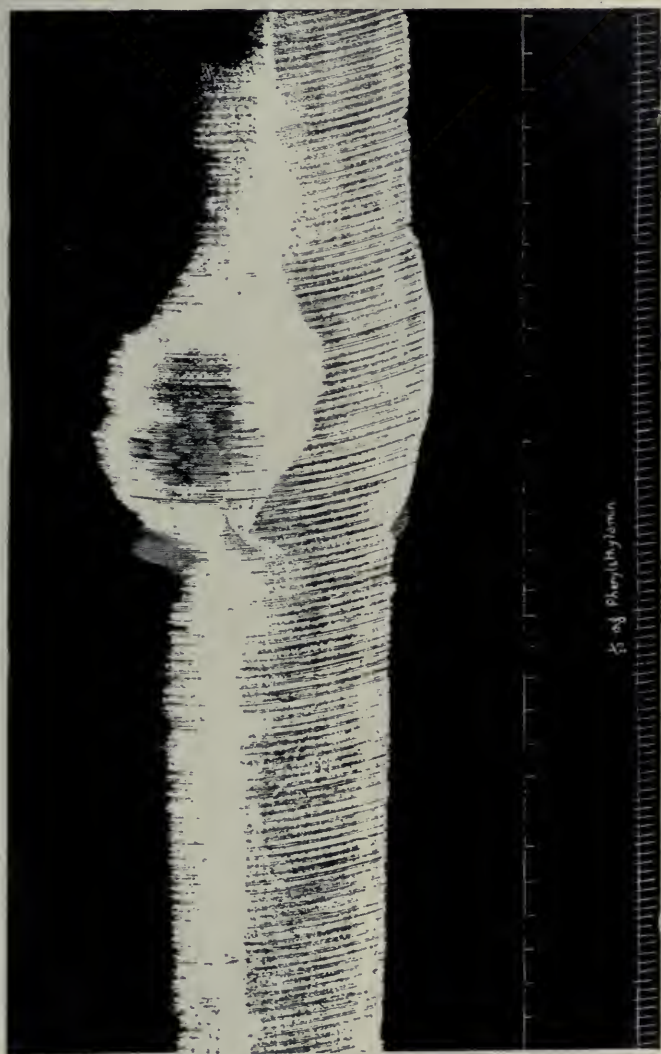


FIG. 3. (See fig. 2.) Phenylethylamin 0.00005 gram.

four to nine times the task which it was accomplishing before the drug was given (see figs. 5 and 6). This heightened activity may last for ten minutes before the normal level again is reached. This is more than a mere recovery from depression. It was at first thought to be the effect of the one-twentieth milligram order of dosage when the concentration passing through the heart became less. But the twentieth of a milligram dose never quickens the rate, furthermore there is here an increase in coronary flow to double or treble the previous flow. Thus we cannot possibly be dealing simply with the effects of returning to a small concentration of phenylethylamin.

The possibility of stimulation followed by paralysis of the vagus, analogous to nicotin action, is excluded by the fact that the slowing phase appears in atropinized hearts of frog, rabbit and cat. Furthermore vagus action is associated with increased rather than decreased amplitude. Finally, if the vagus is paralyzed in the secondary stage, the paralysis must be a very brief one for the whole experiment can soon be repeated. Another explanation suggesting itself for this remarkable increase in activity was the formation by the heart tissues from phenylethylamin of some substance of the nature of epinephrin, the effects of which upon the rabbit's heart are here so closely imitated.

If however we assume, as the primary cause, the abnormally rapid coronary flow, which appears as soon if not sooner than the changes in amplitude and rate, the exaggerated activity of the heart becomes almost self-explanatory. Under the conditions of the experiment the temperature of the heart might become raised by a marked increase in the coronary flow although in many experiments a warm jacket surrounding the heart kept it at a temperature about as high as that of the perfusion fluid. At all events an increased blood supply, within certain limits, will of course augment the cardiac activity.

The probability is therefore that 1 or more mgm. of phenylethylamin depress the heart muscle, and cause a stimulation followed by a temporary paralysis of the coronary blood vessels. The latter effect leads to marked secondary increases in amplitude and rate.

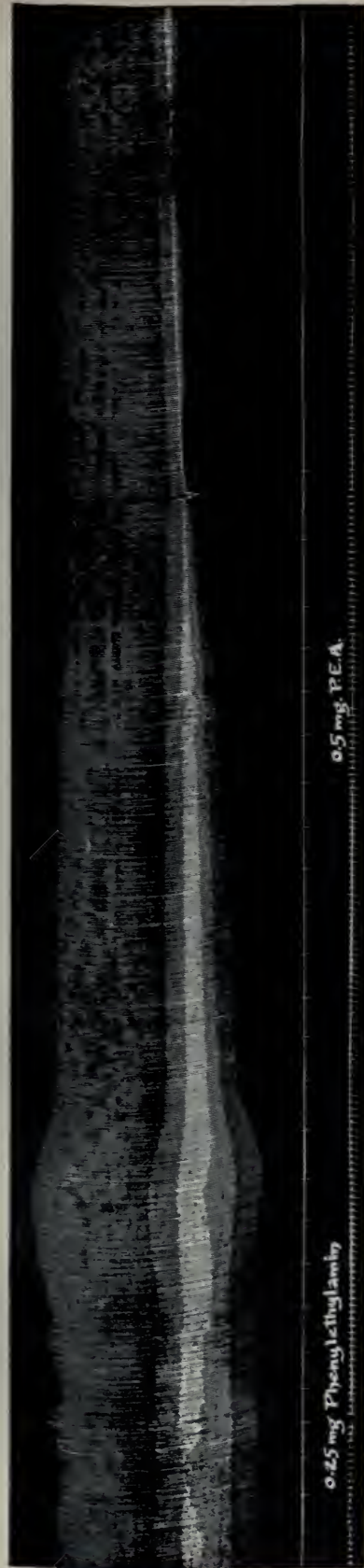


FIG. 4. (See fig. 2.) Phenylethylamine 0.00025, later 0.0005 gram.



FIG. 5. (See fig. 2.) Continuous with figure 4. Phenylethylamine 0.001 gram.



FIG. 6. (See fig. 2.) Phenylethylamine 0.002 gram.

That all of the above described effects are temporary and reversible is shown by the fact that a condition approaching or attaining normal amplitude rate and flow always ultimately recurs, after which the whole series of phenomena can be repeated by reinjection of similar or somewhat larger doses.

Between doses of the order of $\frac{1}{20}$ and 1 mgm. we have met with individual variations in the response, the effects being transitional from the typical small dose effect to the typical large dose effect. Figures 4 and 5 represent a continuous tracing, illustrating successively in one and the same heart a small dose ($\frac{1}{4}$ mgm.) effect, a transitional ($\frac{1}{2}$ mgm.) effect and a large dose (1 mgm.) effect.

Dale and Dixon⁷ have described the action of 1.7 mgm. of isoamylamin, $(\text{CH}_3)_2 \text{CHCH}_2\text{CH}_2\text{NH}_2$, upon the isolated rabbit heart. This appears to correspond in all respects to that of a similar amount of phenylethylamin.

The results of our experiments upon isolated rabbit hearts are summarized in Table I.

(3) ACTION ON INTACT CAT HEARTS

The response to phenylethylamin of the heart under more normal conditions was finally investigated.

Method. Full grown cats were studied by the cardiographic method. In all but one of these experiments a simple "funnel myocardiograph," a modification of Cushny's method for studying changes in the heart volume, was employed.

A small glass funnel was selected, the greatest diameter of which was about equal to that of the heart in diastole. A rubber flange was made for this by cutting off a half inch length of a rubber tube, of somewhat smaller caliber than the greatest diameter of the funnel, and forcing it over the funnel until it overlapped the rim both inside and outside. The pericardium was grasped at three points near the heart's apex and a slit made just admitting the funnel, which was first dipped in oil. Once inside the pericardial sac tension on the stem of the funnel effected

⁷ Dale, H. H. and Dixon, W. E.: Journ. of Physiol., 1909, 39, 25.

TABLE I
Phenylethylamin in isolated rabbit hearts

HEART NO.	PHENYL- ETHYLAMIN- HCL	AMPLITUDE		RATE		CORONARY FLOW	
		(a)	(b)	(a)	(b)	(a)	(b)
		at first	later	at first	later	at first	later
	<i>grams</i>						
1.....	0.000125	increase		no effect		decrease	
2.....	0.000025	increase		no effect		decrease	
1.....	0.00005	increase		no effect		decrease	
4.....	0.00005	increase		no effect		decrease	
5.....	0.00005	increase		no effect		decrease	
4.....	0.0001	increase		no effect		decrease	
1.....	0.0001	decrease	increase to above normal	decrease	increase to above normal	decrease	increase to above normal
3.....	0.0001	decrease	increase to above normal	decrease	increase to above normal	decrease	increase to above normal
3.....	0.00025	increase		no effect		decrease	
1.....	0.00025	decrease	increase to above normal	decrease	increase to above normal	decrease	increase to above normal
3.....	0.0005	decrease	increase to below normal	decrease	increase to below normal	decrease	increase to below normal
1.....	0.0005	decrease	increase to above normal	decrease	increase to above normal	decrease	increase to above normal
6.....	0.0005	decrease	increase to above normal	decrease	increase to above normal	decrease	increase to above normal
1.....	0.001	decrease	increase to above normal	decrease	increase to above normal	decrease	increase to above normal
3.....	0.001	decrease	increase to above normal	decrease	increase to above normal	decrease	increase to above normal
1.....	0.002	decrease	increase to above normal	decrease	increase to above normal	decrease	increase to above normal
3.....	0.002	decrease	increase to above normal	decrease	increase to above normal	decrease	increase to above normal
3.....	0.005	decrease	increase to below normal	decrease	increase to above normal	decrease	increase to above normal
3.....	0.01	decrease		decrease		decrease	

an air tight closure, or if the pericardial slit had been made too long a thread passed around the funnel stem would serve to draw up the pericardium, closing the leak. The stem of the funnel was connected with a Marey tambour. If sufficient permanent tension on the funnel were exerted, only a very small percentage, if any, of the volume change was lost through the flexibility of the pericardial sac. The advantage of this method over the longer glass bell is that there is less crowding of the thoracic contents and no contact of the heart with a rigid foreign body. In experiment 5 a thistle tube cardiometer was employed. Experiments 1-8 were performed upon decerebrate cats, usually lightly curarized. In experiments 9 and 10 instead of decerebration urethan anesthesia was used. Artificial respiration was always employed.

Results. The same general effects were obtained with the intact cat heart as with the isolated rabbit heart. Two essential differences were observed however. Small doses increased the frequency, as well as the amplitude, of the heart beat (as seen in fig. 7). Large doses were never followed by the secondary hyperactivity of the heart noted in the isolated organ. This fact may be attributed to the reduction in blood pressure caused by the primary effect of large doses upon the heart. Owing to this circumstance, the relaxation in the coronary vessels which presumably occurs, cannot result in such an abundant blood supply to the heart as that which is afforded under the continued high artificial pressure of the perfusion apparatus. Furthermore, there is not the opportunity for an increase in the heart's temperature such as may have occurred in the isolated heart when the coronary flow increased.

An increase in rate and amplitude was constantly obtained with doses varying from $\frac{1}{2}$ (the minimal effective dose) to $\frac{1}{2}$ mgm. Amounts of 20 or more mgm. always gave a decrease in amplitude and heart rate. With doses ranging from 1 to 10 mgm. individual variations were noted.

In figure 7 the small dose (0.25 mg.) effect upon the heart is seen, in figure 8 a transitional dose (1 mgm.) effect, in figure 9 the large dose (10 mgm.) effect, with recovery. In experiment

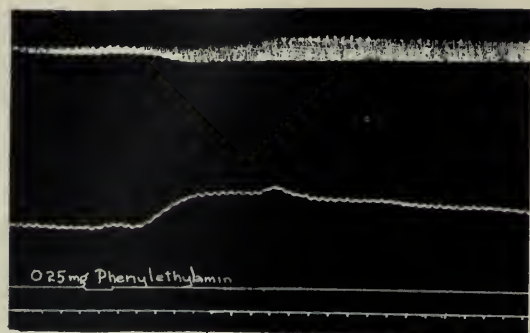


FIG. 7. Experiment 8. Decerebrate cat. Phenylethylamin, $\frac{1}{4}$ mgm. intravenously. 1. Myocardiographic record of heart. 2. Blood pressure. 3. Zero pressure line. 4. Time in 5 second intervals.

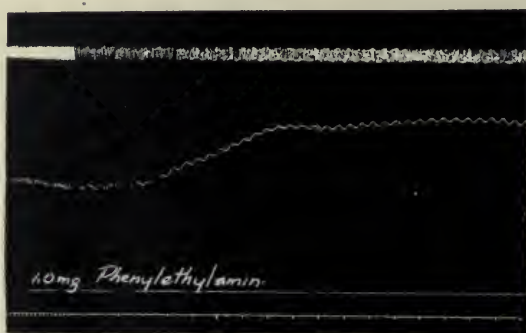


FIG. 8. (Arranged as in figure 7.) Experiment 7. Decerebrate cat. Phenylethylamin, 1 mgm. intravenously.

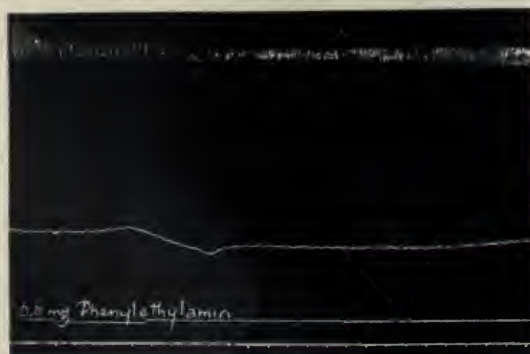


FIG. 9. (Arranged as in figure 7.) Experiment 9. Urethanized cat. Phenylethylamin, 10 mgm. intravenously.

10, 20 mgm. were given after 1 mgm. of atropin sulfat had been injected, and the previously excitable vagus had been proven unresponsive to faradic stimulation. The same degree of decrease in rate and amplitude were obtained as the same dose of phenylethylamin had given in this animal before atropin was injected.

The results of the myocardiograph experiments are summarized in Table II.

SUMMARY AND CONCLUSIONS

1. Phenylethylamin causes in the frog heart a diminution in rate and amplitude in all concentrations from 1:10,000 to 1:500. Smaller concentrations are without any effect, except that 1:15,000 diminishes the amplitude to some extent. This action is a reversible one, for the heart can be restored to its full vigor by re-perfusion, even after it has ceased beating.

2. Perfused through the isolated rabbit heart phenylethylamin caused in small doses (0.0125 mgm. to 0.05 mgm.) a constant increase in the amplitude, without change in the rate. In larger doses (0.5 mgm. to 10 mgm.) decrease in both amplitude and rate are produced. This is followed by a striking secondary effect, amplitude and rate being both increased to far above normal.

Intermediate doses cause transitional effects varying with the individual. A profound diminution in coronary flow results from both small and large doses, but, accompanying these secondary acceleration and augmentation of the beat seen after large doses, there is also an increase of the coronary flow to far above normal. Secondary depression of the muscle of the vessel walls following primary stimulation is the most probable cause for all of the secondary effects.

All of the large dose effects described for phenylethylamin agree substantially with the account by Dale and Dixon of the action upon the isolated rabbit's heart of 1.7 mgm. of isoamylamin.

3. The intact heart of the cat, studied by the myocardiograph responds constantly to small doses (0.2 mgm. to 0.5 mgm.) of

TABLE II
Myocardiograph experiments in cats

EXPERIMENT NUMBER	PHENYL- ETHYLAMIN.	VENTRICULAR AMPLITUDE	PULSE RATE	BLOOD PRESSURE	REMARKS
	<i>grams</i>				
10	0.00001	no change	no change	increase	
10	0.000025	no change	no change	increase	
10	0.00005	no change	no change	increase	
10	0.0001	no change	no change	increase	
10	0.0002	increase	increase	increase	
7	0.00025	increase	increase	increase	
8	0.00025	increase	increase	increase	Before curara (fig. 7)
8	0.00025	increase	increase	increase	After curara
9	0.00025	increase	increase	increase	
7	0.0005	increase	increase	increase	
8	0.0005	increase	increase	increase	
5	0.001	increase	increase	increase	
6	0.001	decrease	increase	increase	
7	0.001	increase	?	increase	(Fig. 8)
9	0.002	increase	increase	increase	
4	0.002	decrease	increase	increase	After 5 mgm.
7	0.002	decrease	no change	increase	
8	0.004	no change	increase	no change	
3	0.005	no change	no change	no change	
4	0.005	decrease	no change	increase	
8	0.005	decrease	decrease	decrease	
2	0.01	increase	decrease	increase	
3	0.01	no change	no change	increase	
4	0.01	no change	no change	increase	
9	0.01	decrease	decrease	decrease	Before atropin (fig. 9)
9	0.01	no change	no change	decrease	After atropin (see 0.025) gm. dose, exp. 9)
3	0.02	decrease	decrease	decrease	
10	0.02	decrease	decrease	decrease	Before atropin
10	0.02	decrease	decrease	decrease	After atropin
8	0.02	decrease	decrease	decrease	
8	0.02	decrease	decrease	decrease	
9	0.025	decrease	decrease	decrease	After atropin
3	0.035	decrease	decrease	decrease	
3	0.04	decrease	decrease	decrease	
3	0.05	decrease	decrease	decrease	
3	0.06	decrease	decrease	decrease	
3	0.075	decrease	decrease	decrease	
2	0.125	decrease	decrease	decrease	Fatal dose

phenylethylamin by an increase in amplitude and rate. (The latter is not affected by similar doses in the isolated rabbit's heart.) Large doses (20 mgm. to 125 mgm.) cause a decrease in both amplitude and pulse rate. Intermediate doses cause varying transitional effects.

4. The blood pressure rises with the increase in heart activity and also where the latter is quite unchanged, showing the vasoconstricting effect. It falls with decreased activity of the heart.

5. The inhibitor effects of phenylethylamin upon the heart do not simulate a stimulation of the vagus nor can they be forestalled by atropin in frog, rabbit or cat.

6. The effects of small and large amounts of phenylethylamin in mammalian as well as in frog hearts are reversible.

7. Phenylethylamin is presumably a cardiac muscle poison, in small amounts stimulating and in large amounts depressing that tissue. In all doses it appears to stimulate constriction of the coronary vessels, large doses resulting in a secondary relaxation.

THE RESPONSE OF THE SURVIVING UTERUS TO MORPHIN AND SCOPOLAMIN¹

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The wide employment of morphin and scopolamin in obstetrics suggests the desirability of more accurate knowledge of the action of these drugs upon the uterus. In this laboratory, therefore, studies have been undertaken upon the response to morphin and scopolamin, given separately as well as together of both the surviving and the living uterus of mammals. The combined action is of further interest because of the numerous experiments of Bürgi,² Kochmann,³ and others, which indicate that morphin and scopolamin in their action upon the central nervous system exhibit a true synergism. By this is meant that the narcotic effect of the combination appears more profound than the algebraic sum of the effects of the same doses given separately. It was thought possible that such an augmentation might be found to exist at points of action outside of the central nervous system.

We have been able to discover no literature upon the action of morphin or of scopolamin upon the uterus in laboratory experiments outside of the work of Kehrer. In his classical studies on the physiology and pharmacology of uterine activity morphin and scopolamin were given rather cursory attention. Kehrer⁴ groups morphin, scopolamin and stovain together and says of their uterine action: "Die drei Narkotika wirken in

¹ A part of the expenses of this research was defrayed from a grant from the Committee on Therapeutic Research, Council of Pharmacy and Chemistry, American Medical Association.

² Bürgi, E.: *Med. Klin.*, 1912, Nn. 50 and 51.

³ Kochmann, M.: *Zeitschr. f. exp. Path. u. Therap.*, 1913, 12, 328.

⁴ Kehrer, E.: *Zentralbl. f. Gynäk.*, 1907, 31, 785.

kleinen Dosen leicht erregend, in hohen Dosen lähmend ein." His experiments were made upon the surviving and living uterus of cat and rabbit. The only details of Kehrer's work on these substances which we have been able to find were in the description of and tracings from experiments showing the action of morphin upon the surviving uterus.⁵ These experiments are too few and too brief to be convincing; successive doses were added at very short intervals.

In the first mentioned paper Kehrer states also that scopolamin caused a slight stimulation of a human uterus. His results with morphin are pointed out as being in accord with clinical experience "dass ganz kleine morphin Dosen die Wehen anzuregen, grössere sie zum Stillstand zu bringen pflegen." The frequent inhibitory effect upon the uterus of morphin-scopolamin narcosis Kehrer says is probably due to the morphin. Morphin and scopolamin act less powerfully upon the uterus than many other substances, and it is for this reason doubtless that more complete studies have not been made.

On the clinical side, Hensen⁶ introduced a rubber balloon into the cervix of parturient women recording the uterine activity by transmitted air pressure. Morphin, he reports, in doses of 5 to 20 mgm. was without effect upon the progress of labor.

That morphin and scopolamin, however, even in small doses, may prolong labor is admitted by the staunchest supporters of this method of anesthesia, for example Gauss.⁷ The great variation in clinical statistics on this question (depending on varying dosage, and a host of other factors), may be seen in a table compiled by Lequeux.⁸

This paper deals with the response of the isolated uterus to morphin and scopolamin applied separately and in combination. Another paper by one of us⁹ describes similar studies upon the living animal.

⁵ Kehrer, E.: Arch. f. Gynäk., 1907, 81, 160.

⁶ Hensen: Arch. f. Gynäk., 1898, 55, 129.

⁷ Gauss, C. J.: Arch. f. Gynäk., 1906, 78, 579.

⁸ Lequeux, P.: L'Obstetrique, 1911, 4 (N. S.), 165.

⁹ Barbour, H. G.: This Journal, 1915, 7, 547.

METHOD

Guinea-pigs and cats, both non-pregnant and pregnant, were employed in this work. The uterus removed from a freshly killed animal was suspended according to Kehrer's method in continuously oxygenated Locke's solution. One-half to one hour was allowed for the rhythmic activity to become regular. The drugs were added by a pipette in the concentrations indicated in the tables. The sulfat of morphin and the hydrobromid of scopolamin (Merck) were employed. The scopolamin solutions were always prepared on the same day on which they were employed.

RESULTS

. Although the appended tables are arranged to separate the two species, as well as the non-pregnant from the pregnant uterus, the action of the drugs is essentially the same throughout.

Action of morphin. (See fig. 1.) When effective this drug gave only an increase in uterine tone, although concentrations as high as 0.1 per cent were brought into direct contact with the uterus. Thus no evidence of a depressed activity was obtained. The amplitude of the individual excursions was usually not affected by morphin. In the largest doses, however, a decrease was noted, always at the expense of the phase of relaxation, a tetanic effect being produced. The frequency of the contractions was usually increased in these cases.

The tone of the uterus was sometimes increased by morphin in concentrations of 0.002 per cent, and always in concentrations of 0.05 per cent or higher.

Action of scopolamin. (See fig. 2.) The tone of the uterus was always increased by scopolamin in concentrations of 0.005 per cent or greater. In the case of one pregnant guinea-pig uterus both amplitude and rate were increased by a concentration of 0.001 per cent. The highest concentration of scopolamin used (0.06 per cent) gave an increase in tone and rate, the amplitude of the contractions being here diminished. In general the effects of scopolamin upon the isolated uterus are the same as

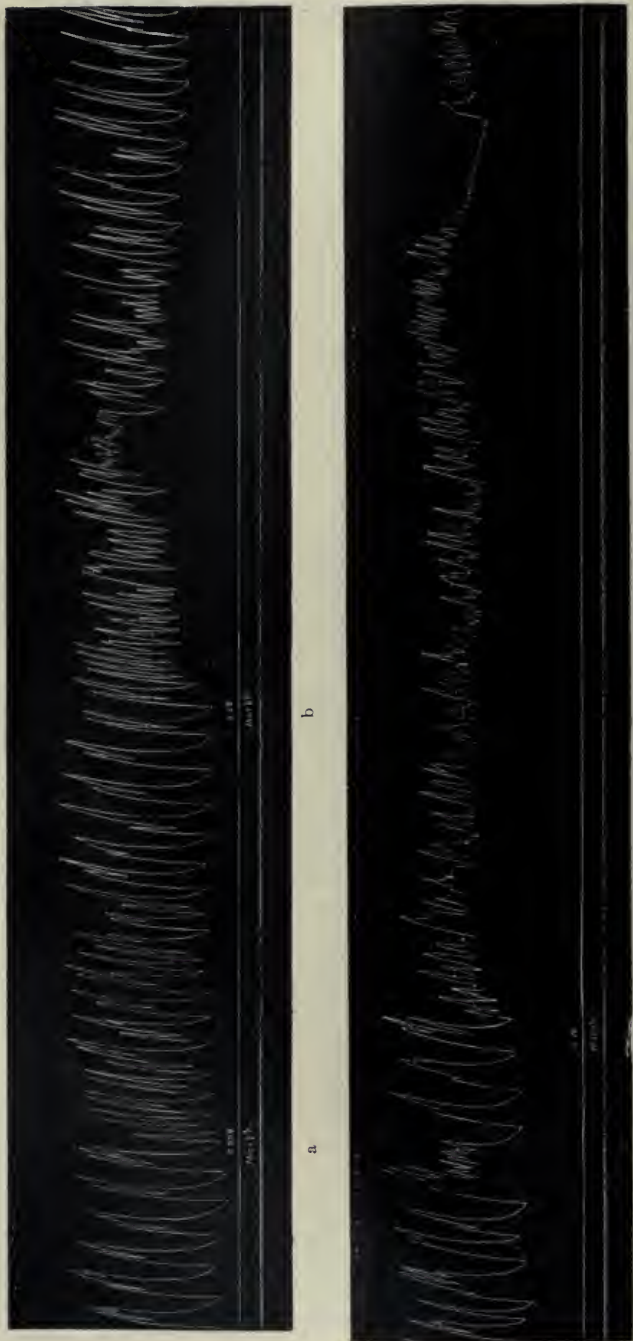


FIG. 1. (Experiment XI.) Effect of morphin upon isolated pregnant cat uterus.
 (a) 0.002 per cent (0.004 g. in 200 cc. Locke's solution).
 (b) 0.01 per cent (0.02 g. in 200 cc. Locke's solution).
 (c) 0.05 per cent (0.1 g. in 200 cc. Locke's solution).
 Time in 5 second intervals. (Lowest line.)

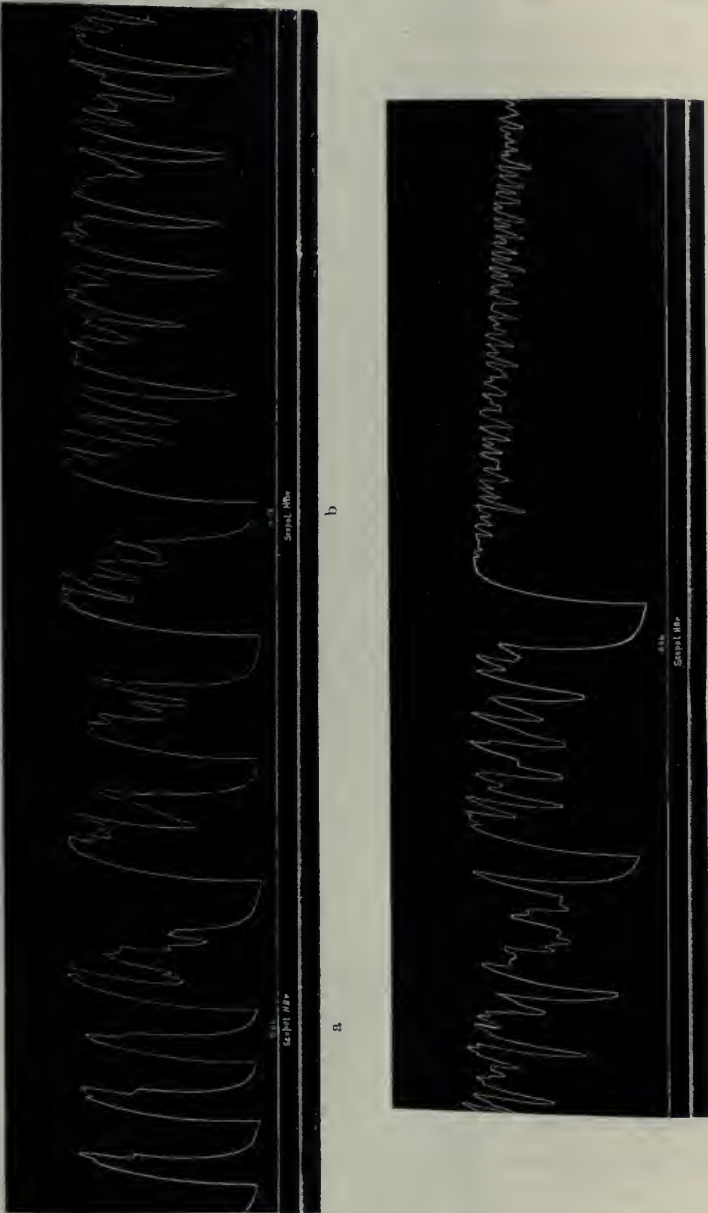


Fig. 2. (Experiment XVII.) Effect of scopolamin upon isolated non-pregnant guinea pig uterus.
(a) 0.01 per cent (0.01 g. in 100 cc. Locke's solution).
(b) 0.03 per cent (0.03 g. in 100 cc. Locke's solution).
(c) 0.06 per cent (0.06 g. in 100 cc. Locke's solution).
Time in 5 second intervals. (Lowest line.)

those of morphin but the former alkaloid is about ten times as powerful as the latter in this activity.

Action of morphin and scopolamin together. (See fig. 3.) The combined action of these drugs differed, in our experiments, in no essential way from their individual action. Where amounts of scopolamin and morphin were given together, of which the sum, judged from their separate effects, was subminimal, there was also no effect from the combination. Thus the lowest effective dosage was 0.01 per cent morphin plus 0.001 per cent scopolamin; this amount (or even less) of morphin given alone had sometimes yielded as great an effect, and at other times proved ineffective. From a careful comparison of all of our experiments one can find no evidence that either drug augments or diminishes in any way the effects of the other. Synergism and antagonism both appear to be lacking.

In fifty-nine experiments on organs from seventeen different animals no single instance of diminution in tone or rate of the uterus could be obtained either with scopolamin or with morphin. The only decreases in amplitude were associated with tetanic-like increase in tone. That doses up to 0.1 g. of morphin in 100 cc. of fluid gave only an increase in tone seems to preclude the possibility of this drug ever producing during life a relaxation by direct action on the uterus. 0.1 g. intravenously given to cats having 100 cc. of circulating blood is not only fatal or nearly so, but only a fractional part of the dose would have opportunity for prolonged contact with the uterus.

The deduction that prolongation of labor by either or both of the alkaloids here considered is due to some action other than a direct one upon the uterus is borne out by the further work upon living animals above referred to.

CONCLUSIONS

The following are the results of experiments on surviving pregnant and non-pregnant uterus of guinea-pig and cat:

1. Morphin, in concentrations of from 0.05 per cent to 0.1 per cent and sometimes as low as 0.002 per cent, stimulates the isolated uterus to an increase in tone.



FIG. 3. (Experiment V.) Effect of morphin plus scopolamin upon isolated pregnant guinea pig uterus.

- (a) 0.01 per cent morphin (0.01 g. in 100 cc. Locke's solution).
 0.001 per cent scopolamin (0.001 g. in 100 cc. Locke's solution).
 (b) 0.05 per cent morphin (0.05 g. in 100 cc. Locke's solution).
 0.005 per cent scopolamin (0.005 g. in 100 cc. Locke's solution).

Time in 5 second intervals. (Lowest line.)

2. Scopolamin, in concentrations of 0.005 per cent to 0.06 per cent, and sometimes as low as 0.001 per cent, increases the tone of the isolated uterus. In this respect, therefore, it appears to be about ten times as powerful as morphin.

3. The inhibitory action upon the tone of the uterus described by Kehrer for large doses, could be obtained with neither of the above substances. Very high concentrations of either tend to produce a tetanic condition of the organ.

4. No synergism or antagonism could be demonstrated in the direct action of these drugs upon the uterus.

TABLE I
Non-pregnant guinea pig uterus

EXPERIMENT	LOCKE'S SOL.	DRUG	CONCENTRATION	UTERINE TONUS*	AMPLITUDE OF CONTRACTIONS*	RATE OF CONTRACTIONS*
	cc.	gram	per cent			
I Mar. 2, '15	100	Morph. 0.002 Morph. 0.01 Morph. 0.05	0.002 0.01 0.05	0 ++ 0	0 0 0	0 + 0
II Mar. 4, '15	100	Morph. 0.002 Morph. 0.01 Morph. 0.05	0.002 0.01 0.05	+ ++ ++	0 0 (-)	0 (-) 0
					(Went into tetanus)	
IX Mar. 25, '15	100	Scopol. 0.00025 Scopol. 0.001 Scopol. 0.005	0.00025 0.001 0.005	0 0 ++	0 0 (-)	0 0 +
XVII Apr. 24, '15	100	Scopol. 0.01 Scopol. 0.03 Scopol. 0.06 Washed and changed solution Morph. 0.1	0.01 0.03 0.06 0.1	+ ++ +++ ++	0 (-) (-) (-)	(-) + ++ +

* + = increase. (-) = decrease. 0 = no effect.

TABLE II
Pregnant guinea-pig uterus

EXPERIMENT	LOCKE'S SOL.	DRUG	CONCENTRATION	UTERINE TONUS	AMPLITUDE OF CONTRACTIONS	RATE OF CONTRACTIONS
III	cc.	gram	per cent			
Mar. 9	100	Morph. sulf. 0.002	0.002	0	0	0
		Morph. sulf. 0.01	0.01	0	0	0
		Morph. sulf. 0.05	0.05	++	(-)	+
		Washed and changed Locke's solution				
		Scopol. HBr. 0.00025	0.00025	0	0	0
		Scopol. HBr. 0.001	0.001	+	+	0
		Scopol. HBr. 0.005	0.005	0	++	0
IV						
Mar. 11	100	Scopol. HBr. 0.00025	0.00025	0	Increased contraction	(-)
		Scopol. HBr. 0.001	0.001	0	Increased contraction with no increase in relaxation	(-)
		Scopol. HBr. 0.005	0.005	0	0	0
		Washed and changed Locke's solution				
		Morph. sulf. + Scopol. HBr. 0.01+0.001	0.01+0.001	++	+	+
V						
Mar. 13	100	Morph. + Scopol. 0.002+0.00025	0.002+0.00025	0	+	0
		0.01 +0.001	0.01 +0.001	0	0	0
		0.05 +0.005	0.05 +0.005	++	(-)	0
VI						
Mar. 16	100	Morph. sulf. 0.002	0.002	0	0	0
		Morph. sulf. 0.005	0.005	0	0	0
		Morph. sulf. 0.01	0.01	+	+ (slight)	0
		Morph. sulf. 0.02	0.02	++	+	0
VII						
Mar. 20	100	Morph. + Scopol. 0.005+0.001	0.005+0.001	0	0	0
		Washed and changed Locke's solution				
		0.005+0.005	0.005+0.005	0	+	0
VIII						
Mar. 23	100	Scopol. HBr. 0.00025	0.00025	0	0	0
		Scopol. HBr. 0.001	0.001	0	0	0
		Scopol. HBr. 0.005	0.005	+	0	(-)
		Washed and changed solution				
		Morph. + Scopol. 0.005+0.001	0.005+0.001	0	0	0

TABLE III
Non-pregnant cat uterus

EXPERI- MENT	LOCKE'S SOL.	DRUG	CONCENTRATION	UTERINE TONUS	AMPLITUDE OF CON- TRACTIONS	RATE OF CONTRAC- TIONS
	cc.	gram	per cent			
X Mar. 27	100	Morph. 0.002	0.002	0	0	0
		Morph. 0.01	0.01	0	0	0
		Morph. 0.05	0.05	+	(-)	0
					(Went into tetanus)	
XVI Apr. 20	200	Morph. + Scopol.				
		0.004 + 0.001	0.002 + 0.0005	0	0	0
		0.02 + 0.002	0.01 + 0.001	+	+	0
			Washed and changed solution			

TABLE IV
Pregnant cat uterus

EXPERIMENT	LOCKE'S SOL.	DRUG	CONCENTRATION	UTERINE TONUS	AMPLITUDE OF CONTRACTIONS	RATE OF CONTRACTIONS
	cc.	gram	per cent			
XI (Early pregnancy) Mar. 30	200	Morph. 0.004	0.002	0	0	+
		Morph. 0.02	0.01	++	(-)	++
		Morph. 0.1	0.05	++	(-)	++
XII Apr. 6	200	Morph. 0.004	0.002	0	0	+
		Morph. 0.02	0.01	0	0	+
		Morph. 0.1	0.05	+	(-)	+
		Soon went into tetanus				
XIII (Early pregnancy) Apr. 6	200	Scopol. 0.0005	0.00025	0	+	(-)
		Scopol. 0.002	0.001	0	0	0
		Scopol. 0.01	0.005	+	0	+
XIV (Late pregnancy) Apr. 7	200	Scopol. 0.005	0.0025	0	0	0
		Scopol. 0.002	0.001	0	0	(-)
		Scopol. 0.01	0.005	++	(-)	+
		Washed and changed solution				
		Morph. +Scopol. 0.02+0.002	0.01+0.001	+	(temporary)	0
				++	0	0
		0.1 +0.002	0.05+0.001			
XV (Late pregnancy) Apr. 8	200	Morph. 0.004	0.002	0	0	0
		Morph. 0.02	0.01	0	0	0
		Morph. 0.1	0.05	+	0	+
		Washed and changed solution				
		Morph. +Scopol. 0.1+0.002	0.05+0.001	+	0	+

THE ACTION OF CAFFEIN AND OF EPINEPHRIN UPON THE VAGUS NERVE¹

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Both caffein and epinephrin are capable of accelerating the heart, but this effect is frequently masked by the central vagus stimulation which either of the two drugs can produce.

In hearts freed from the control of central nervous system, however, the increase in pulse rate is constantly obtained. This acceleration is due chiefly to stimulation of the "excitomotor apparatus" of the heart. Cushny and Van Naten² showed this to be true of caffein, for adequate doses of this drug given to atropinized animals in which the accelerator nerve trunks had been divided always increased the heart rate. However it does not appear to have been settled whether or not caffein has any action upon the peripheral terminations of the vagus in the heart. According to H. Fredericq³ these endings, in the dog's heart, are rendered more excitable by caffein.

In the case of epinephrin, on the other hand, a temporary depression of the vagus endings in the heart is shown by the work of Cybulski,⁴ Gourfein,⁵ Langley⁶ and others, upon mammals.

The results of a number of observations upon frogs bearing upon the question of caffein and epinephrin action upon the vagus endings in the heart are here presented.

¹ The expenses of this research were defrayed from the Francis E. Loomis Fund of the Yale University School of Medicine.

² Cushny, A. R., and Van Naten, B. K.: *Arch. Internat. d. Pharmacodynamie*, 1901, 9, 169.

³ Fredericq, H.: *Arch. Internat. d. Physiol.*, 1913, 13, 107.

⁴ Cybulski, N.: *Anzeiger d. Akad. d. Wissensch. in Krakau*, 1895, p. 82.

⁵ Gourfein: *Compt. Rend. Acad. des Sci.*, 1895, 121, 311.

⁶ Langley, J. N.: *Journ. of Physiol.*, 1901, 27, 245.

The experiments were similar to those described by Loewi⁷ in his pharmacological studies of the frog vagus. The heart of the pithed animal was perfused through a cannula inserted in the inferior vena cava. The different solutions were perfused from Mariotte bottles in which equal pressures were maintained. The carotid arteries were cut, to eliminate vascular resistance. The vagus was placed on fine copper electrodes, suitable precautions being taken against the drying out of the nerve or the miscarriage of the current. The heart action was recorded by the usual suspension method. The Ringer's solution employed contained NaCl, 0.7 per cent; CaCl₂, 0.026 per cent; KCl, 0.03 per cent. This was used for all perfusions, the drugs being added to it to make the concentrations given in the tables.

The following points are brought out by the accompanying tables:

TABLE I
Caffein perfusion

NUMBER OF EXPERIMENT	RINGER'S SOLUTION	CAFFEIN + RINGER'S SOLUTION			DURING RE-PERFUSION WITH RINGER'S SOLUTION		
	Minimal stimulus causing vagal standstill	Concen- tration	Stimulus	Effect	Stimulus	Effect	Number of seconds after caffein perfusion
		<i>per cent</i>					
1	8.0*	0.01	8.0	Stop			
2	10.0	0.02	10.0	Stop	10.5	Stop	380
3	9.0	0.05	9.0	Slowing			
4	9.0	0.05	6.5	None	8.0	Stop	15
5	6.5	0.05	0.0	None	5.5	Stop	600
6	5.5	0.05	2.0	None	4.5	Slowing	540
7	8.0	0.05	0.0	None	8.0	Stop	120
8	8.0	0.05	4.0	None	8.0	Stop	?
9	9.5	0.05	8.5	Slowing	8.5	Stop	43
10	10.0	0.10	0.5	None	8.0	Stop	390

* These numbers denote the distance in centimeters that the secondary coil was withdrawn from the primary coil.

1. Both caffein and epinephrin when perfused through the frog's heart decrease markedly the response to faradic stimulation of the vagus.

⁷ Loewi, O.: Arch. f. exp. Path. u. Pharm., 1912, 70, 323.

2. To produce this effect the minimal adequate concentration of caffein lies between 0.02 per cent and 0.05 per cent, of epinephrin, between 0.0005 per cent and 0.001 per cent.

TABLE II
Epinephrin perfusion

NUMBER OF EXPERIMENT	RINGER'S SOLUTION	EPINEPHRIN + RINGER'S SOLUTION			DURING RE-PERFUSION WITH RINGER'S SOLUTION		
	Minimal stimulus causing vagal standstill	Concentration	Stimulus	Effect	Stimulus	Effect	Number of seconds after epinephrin perfusion
		<i>per cent</i>					
11	8.5	0.0003	8.0	Stop	8.0	Stop	575
12	11.5	0.0005	9.0	Stop	9.0	Stop	140
13	9.0	0.0005	6.5	Stop	0.5	None	500
14	8.0	0.001	0.5	Slowing	5.0	Stop	75
15	7.0	0.001	0.0	None	0.1	Stop	415
16	7.0	0.001	5.0	None	6.5	Stop	210
17	8.0	0.001	4.0	Slowing	4.0	Stop	240
18	9.5	0.001	2.0	None	1.0	None	510
19	8.5	0.001	0.0	None			

TABLE III
Averages from Tables I and II

NUMBERS OF EXPERIMENTS	AVERAGE MINIMAL STIMULUS CAUSING VAGAL STANDSTILL WITH RINGER'S	DRUG AND CONCENTRATION	AVERAGE MAXIMAL STIMULUS NOT CAUSING VAGAL STANDSTILL DURING DRUG PERFU-SION	AVERAGE MINIMAL STIMULUS CAUSING VAGAL STANDSTILL DURING RE-PERFUSION WITH RINGER'S
		<i>per cent</i>		
3, 4, 5, 6, 7, 8, 9	7.9	Caffein 0.05	4.3	7.6 (5 experiments)
14, 15, 16, 17, 18, 19	8.0	Epinephrin 0.001	0.9	3.9 (4 experiments)

3. There is after caffein a more complete recovery of vagus sensitivity than after epinephrin.

The question arises whether such results demonstrate a real vagus depression or merely an over-shadowing of vagal inhi-

bition by an increase in the irritability of the heart muscle. Dixon's⁸ statement that epinephrin does not increase the irritability of heart muscle, but that caffeine does so, accords with other known characteristics of epinephrin (affinity for myoneural junctions) and of caffeine (a muscle poison). A true depression of the vagus nerve would therefore appear to be the explanation for our results with epinephrin. The effects of caffeine however may be due in part to vagus depression, although probably dependent to some extent upon increased irritability of the heart muscle.

Some further experiments with caffeine were corroborative of the above results. For example, caffeine perfusion of hearts previously perfused with epinephrin was performed twice, decrease in vagal response being obtained in both cases. The same result was obtained in three experiments in which caffeine was applied to the heart externally only (a few drops of 0.1 per cent or of 1.0 per cent solution).

Injection into the lymph sacs of two frogs of about 0.03 gram of caffeine each gave negative results. Experiments in which sheep serum was substituted for Ringer's solution were found impracticable because of the frequent occurrence of heart block.

Figures 1 and 2 illustrate the effect of caffeine upon the vagus.

⁸ Dixon, W. E.: *Journ. of Physiol.*, 1903, 30, 97.

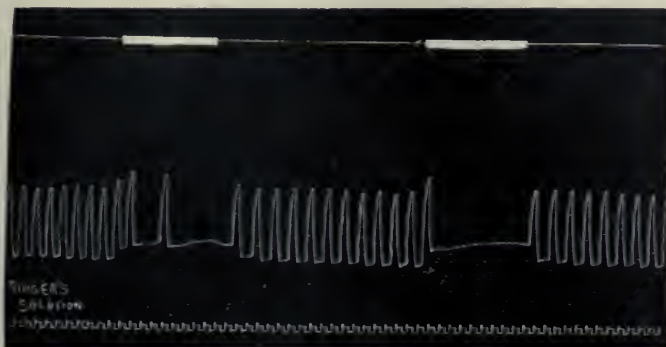


FIG. 1. (Experiment 4.) Perfusion of frog heart with Ringer's solution. First stimulation of vagus (top line) with secondary coil at 9.5, second stimulation, 9.0. Third line; time in seconds.



FIG. 2. (Same experiment.) Begins during caffein—Ringer perfusion. First two stimulations of vagus (7.5 and 6.5 respectively) ineffective. Third stimulation (8.0) effective after pure Ringer's solution had been substituted at point indicated.

MORPHIN AND SCOPOLAMIN ACTION UPON THE INTACT UTERUS¹

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A study of the action of morphin and scopolamin upon the isolated mammalian uterus² shows that both of these drugs increase the tone of that organ. This is true of all concentrations adequate to affect the uterus at all.

In this paper will be described a method of studying uterine activity in the intact animal, and the results of its application to the morphin and scopolamin question.

METHOD

Although the intact uterus has frequently been made the object of study, while directly exposed to room temperature and air, a few trials convince one that a record of uniform uterine action of long duration is extremely difficult to obtain under these conditions. The occasional application of warm saline causes irregular changes in tone and it is hardly more satisfactory to attempt the employment of an evenly regulated saline drip.

The method used in this work was suggested by Paul Trendelenburg's³ device for studying the movements of the intestines *in situ*. The uterine movements *in situ* are simpler to record than are those of the small intestine because the lower end

¹ A part of the expenses of this research was defrayed from a grant from the Committee on Therapeutic Research, Council of Pharmacy and Chemistry, American Medical Association.

² Barbour, H. G. and Copenhaver, N. H.: *This Journal*, 1915, 7, 529.

³ Trendelenburg, P.: *Zeitschr. f. Biol.*, 1913, 51, 67.

of the uterus is by nature held fixed to the framework of the body. The procedure is as follows:

A female cat is etherized and transferred to a warm table. Both carotids are ligated, a tracheotomy performed and artificial respiration instituted. The animal is next pithed, either by a probe passed through the foramen magnum destroying the medulla and the cranial end of the spinal cord or by decerebration through the orbits. An injection cannula is inserted in one femoral vein and the blood pressure is recorded from the carotid artery. Light curarization is employed.

An abdominal incision is made in the median line directly above the pubis, for a distance of about 4 cm. After the contents of the bladder have been expressed the two horns of the uterus are tied together very near their ovarian ends, the blood vessels being included in the ligature. Just below this another ligature is made, with a longer thread, by which, after transection between the ligatures almost the entire organ can be brought into the glass cylinder in which it is to be suspended. This cylinder measures 9 x 2.5 cm. and fits at its lower end into a grooved rubber cork. The abdominal wall is slipped into this groove, and one or two stitches will now make the peritoneal cavity fluid-tight except for the cylinder, where the protecting fluid is next poured in.

Instead of surrounding the uterus with saline a bland oil is employed on account of its poorer heat conduction. For this purpose warm albolene (suggested by Prof. Y. Henderson) is used. The oil fills the abdominal cavity and completely covers the uterus in the cylinder. It is kept in rhythmical motion by the movements of the diaphragm (from the respiration pump), and as it rises and falls in the cylinder the uterus continually benefits by the higher temperature of the abdominal cavity.

The cylinder is next enclosed in an asbestos cover or other further means of sparing heat, and the whole is held firmly by a clamp. In applying the clamp upward tension on the cylinder promotes a freer communication with the abdominal cavity. Great care is taken that the uterus be kept from contact with the cylinder at any point, the top of the latter should therefore incline slightly towards the animal's head. The thread is passed over pulleys to a lightly weighted recording lever (magnification $6\frac{1}{2}$ times).

In pregnant animals only the body of the uterus and that portion of the horns which lies below the foetal enlargements is employed.

In the present work a control injection of epinephrin was usually made at the end of an experiment, characteristic responses always being obtained.

The sulfat of morphin and hydrobromid of scopolamin (Merek) were employed in this work. In those instances in which the scopolamin solution was not freshly prepared a solution in 10 per cent mannit⁴ (not over a few weeks old) was used. Ten per cent mannit given alone did not effect uterine activity in the cat.

RESULTS

The results of the experiments upon decerebrate cats are tabulated below.

TABLE

Effects of morphin and scopolamin upon the uterus of decerebrate cats

+ = increase in tone, (-) = decrease in tone, 0 = no effect.

EXPERIMENT NUMBER	METHOD OF PITHING	CONDITION OF UTERUS	MORPHIN		SCOPOLAMIN		MORPHIN AND SCOPOLAMIN		
			Grams	Result	Grams	Result	Grams M.	Grams S.	Result
3	Foramen magnum	Non-pregnant			0.00002	+			...
					0.00002	0			
					0.0001	+			
					0.00025	0			
			0.001	0			0.001	0.0001	0
					0.001	+			
4	Foramen magnum	Non-pregnant			0.003	0			
					0.01	0			
			0.05	+					
			0.005	0					
15	Foramen magnum	Non-pregnant	0.001	0					
			0.003	0					
			0.005	0					
			0.01	0					
			0.05 ^a	(-)					
			0.02	0					
			0.06 ^b	(-)					

^a = resulted in complete circulatory failure.

^b = resulted in a 25 mm. fall in blood pressure.

⁴ Cf. Straub, W.: Münch. med. Wochenschr., 1913, Nr. 41, 2279.

TABLE—Continued

EXPERIMENT NUMBER	METHOD OF PITHING	CONDITION OF UTERUS	MORPHIN		SCOPOLAMIN		MORPHIN AND SCOPOLAMIN		
			Grams	Result	Grams	Result	Grams M.	Grams S.	Result
17	Foramen magnum	Non-pregnant	0.02 0.02	+ +	0.005 0.005 0.005	(-) (-) (-)	0.01	0.0025	0
19	Foramen magnum	Pregnant	0.02	0	0.005	+			
21	Orbits	Non-pregnant			0.001 0.005 0.01 0.005	+ + + 0			
22	Orbits	Non-pregnant	0.02 0.06 0.1	0 0 0	0.005 0.01 0.02 0.0005 0.001 0.005 0.05	0 0 0 0 0 0 0			
23	Orbits	Non-pregnant	0.02 0.06	+ +	0.005 0.01 0.02 0.05	+ 0 + +	0.02	0.02	+
24	Orbits	Non-pregnant	0.01 0.02 ^a	0 (-)					
25	Orbits	Pregnant	0.02	0	0.01 0.03	0 0	0.02	0.02	0
26	Foramen magnum	Non-pregnant	0.02	0			0.02	0.01	0

^a = resulted in complete circulatory failure.

The various doses were in each experiment injected in the order given in the above table. An interval of half an hour or longer was allowed between any two injections.

Action of morphin. The uterus in some animals refused to respond to either alkaloid, even in very large doses. For example, in experiment 22, one decigram of morphin failed to alter tone or rhythm. When this injection was made nearly a decigram each of morphin and scopolamin had already been given without effect.

The increase in tone described for the isolated uterus⁵ was however seen in a number of experiments after the injection of 0.02 gram of morphin. This was the smallest effective dose.

That morphin never directly inhibited the tone of the uterus should be emphasized. There was a relaxation following three injections of morphin all of which caused serious depression of the blood pressure. In experiment 15, 0.05 gram caused complete failure of the circulation, which was restored however by epinephrin; later 0.06 gram caused a fall of 25 mm. of mercury in the blood pressure. Both of these injections resulted in marked inhibition of uterine tone and lowered frequency of contractions. In experiment 24, 0.02 gram proved fatal and of course the uterus relaxed and its activity soon ceased. All of the other injections of morphin have failed either to lower the blood pressure or inhibit the uterus.

An increase in tone, inconstant, and obtained with comparatively large doses (such as interfere markedly with natural respiration in the cat), is the only effect on the uterus to be definitely ascribed to morphin.

Morphin on intact rabbit uterus. Additional experiments have been made upon rabbits under paraldehyde. The same method of recording uterine action was employed. To 0.02 gram of morphin three animals all responded by an increase of uterine tone.⁶

⁵ Barbour and Copenhaver: l. c.

⁶ For these observations I am indebted to three groups of the class in experimental pharmacology. (They were allowed to expect the opposite effect.)

Action of scopolamin. Scopolamin also was frequently given in large doses without result. Some animals gave no response to this potent alkaloid even when several centigrams were given. On the other hand the increase of tone which we have described for the isolated uterus was seen in a number of cases. This is however, as with morphin, a temporary phenomenon. (See fig. 1.)

Only one cat (experiment 17) exhibited relaxation of the uterus from scopolamin. This occurred each time after four successive doses of 0.005 gram each. It was always of fifteen or twenty minutes' duration but never associated with any diminution of rate or amplitude of the contractions being simply a gradual

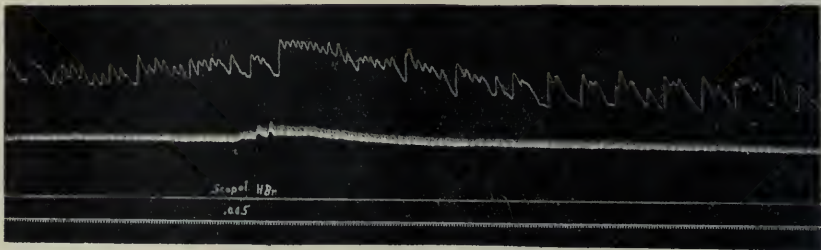


FIG. 1. Experiment 23. Non-pregnant cat. Pithed through orbits. Slight temporary increase of uterine tone after 5 mgm. of scopolamin. No other drugs except curara had been given. 1. Uterine contractions (upward stroke.) 2. Blood pressure. 3. Base line. 4. Time in 5 second intervals.

sinking and rising again of the tonus. An unusually marked increase in heart rate and rise in blood pressure accompanied each of these injections. We were unable to repeat this phenomenon in other cats, and are convinced that, in therapeutic doses at least, scopolamin produces no effects of consequence upon the uterus.

Action of morphin and scopolamin together. When the two drugs were injected together although five animals were tested, only one positive result was obtained. This was an increase in tone such as might have been expected from either drug alone in the same dose. The tracing from a pregnant uterus shown in

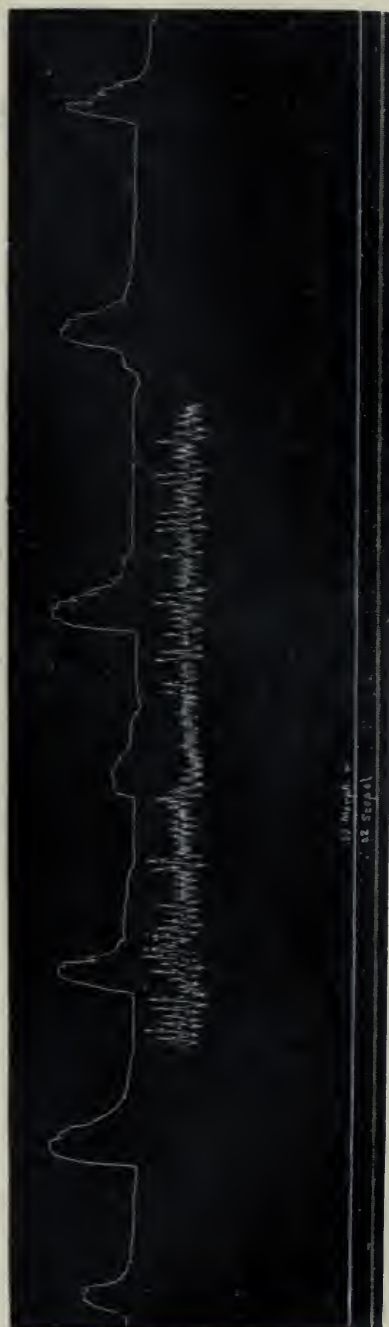


FIG. 2. (Arrangement as in fig. 1.) Experiment 25. Pregnant cat. Pithed through orbits. Curara. Injection of morphin and scopolamin (0.02 gram each) without result.

figure 2 is typical of the experiments in which morphin and scopolamin were given together. Uterine contractions and blood pressure both remained practically constant.

DISCUSSION

From these results and from the above mentioned work on the isolated uterus it will be seen that the only change in uterine tone due directly to either drug (with the possible exception of scopolamin in one living cat) has been not a decrease but an increase. Diminutions in the frequency or amplitude of the contractions have also been insignificant and rare.

It must not be forgotten, however, that there is abundant clinical evidence that morphin may delay the progress of labor. This is, I believe, to be attributed chiefly, if not entirely, to the action of morphin upon the cerebrum.

This factor is of course excluded when decerebrate animals are used. But even with non-decerebrate animals (the rabbits) no diminution in uterine tone was observed. We had in the latter case, however, no normal cerebrum for our control period, because of the influence of the necessary anesthetic. The explanation of the delay in labor resulting from morphin and scopolamin undoubtedly lies simply in the general quieting and narcotic action of these drugs, in the same way that it is recognized that normal sleep diminishes the frequency of the uterine contractions.

Part of a protocol from experiments upon a parturient female dog (weighing 10 kilograms) will serve to illustrate the depressing action of morphin upon the labor pains of the previously alert animal. The "contractions" referred to were obvious from external observation.

January 15, 1915. 3.25 p.m. Found with one still-born pup, others not yet delivered.

3.30. Given 0.05 gram p. oxyphenylethylamin subcutaneously; active contractions at 2 or 3 minute intervals resulted. Respiration 7 x 4.

3.50. Contractions continue. Given 0.02 gram *morphin sulfat* subcutaneously. Vomited twice. Contractions soon ceased.

- 4.00. Asleep.
- 4.20. Respiration 5 x 4. Sound asleep.
- 4.37. Intravenous injection of 0.1 gram p. oxyphenylethylamin.
- 4.45. No contractions yet.
- 4.57. Injection of p. oxyphenylethylamin repeated. No signs of uterine action, nor have there been any since morphin was given.
- 5.30. There have been no contractions.

In this case it is difficult to believe that the morphin did not cause the cessation of uterine activity, which was synchronous with entrance upon morphin narcosis. Even the oxytocic principle from ergot, p. oxyphenylethylamin, appeared unable to combat the uterine inertia arising from the cerebral action of morphin.

CONCLUSIONS

1. The contractions of the uterus in living animals can be recorded conveniently by the method described in this paper. The organ is suspended in a glass cylinder made continuous with the peritoneal cavity. The whole is filled with warm oil kept gently circulating by the movements of the diaphragm.

2. Neither morphin or scopolamin cause profound changes in the activity of the pregnant or non-pregnant uterus of decerebrate cats. A temporary increase in tone such as described for the isolated organ is often seen in the case of both drugs. Morphin in decerebrate cats may cause indirectly a relaxation of the uterus if a marked depression of the circulation be produced.

3. Morphin (0.02 gram) increases the tone of the uterus of rabbits under paraldehyde.

4. Doses up to 0.02 gram each of morphin and scopolamin given together possess little or no action upon the uterus.

5. The statement that large doses of these narcotics inhibit the activity of the uterus is not applicable to any direct action of morphin or scopolamin upon this organ. The delay in labor produced by either or both of these drugs is probably due entirely to their cerebral action.

THE ABSORPTION OF POTASSIUM IODID BY PERFUSED THYROID GLANDS AND SOME OF THE FACTORS MODIFYING IT

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The data included in this report have to deal with the question: Have the surviving thyroid cells *in vitro* a specific affinity for iodine? It is based upon the conviction that there is abundant proof that the thyroid cells *in vivo* exhibit this specific affinity for iodine, and that this biological characteristic is utilized for the purpose of elaborating a physiological secretion highly important to the host rather than that it is utilized for the purpose of rendering the iodine of the body inert and harmless. A few of the more general facts upon which this conviction is based may be referred to at this time. Thus, it has been proved that the gland readily acquires iodine from any form and manner in which it has so far been administered. The amount taken up is relatively so great that maximum thyroid effects are produced by exceedingly small quantities. The quantity taken up and the rapidity of storage have been found to depend upon the degree of saturation of the thyroglobulin existing at the time of its administration. That is, the lower the original iodine content, the more rapidly it is stored from a given intake, or if expressed in anatomical terms, the ability of the gland to store iodine varies directly with the degree of active hyperplasia. No other body tissue exhibits such characteristics. There is a fairly constant per cent of iodine necessary for normal gland structure in mammals, and also a relatively constant maximum per cent for mammalian thyroid tissue. As could be inferred from the relatively constant maximum iodine percentage and the relatively

constant minimum per cent associated with normal gland structure, it is found that iodine invariably involutes the physiological hyperplasias of the gland to their colloid or quiescent state; also that it prevents this compensatory overgrowth both in the intact gland and in the stumps of partially removed glands, which otherwise would undergo hyperplasia. This action is exhibited with amounts of iodine so small that many observers are still unable to associate such quantities with any physiological action. However, we have repeatedly seen amounts as low as $\frac{1}{2}$ mgm. of iodine administered by mouth at weekly intervals wholly prevent thyroid overgrowth in pups, while other pups of the same litter living in the same kennel developed well marked goitres.

Oswald had shown that the lowered iodine content was not associated with any demonstrable reduction in the quantity of thyroid protein (thyroglobulin) with which the iodine is, for the most part, bound. All late work has confirmed this observation, and in addition established the facts that the only known and highly specific physiologic and pharmacologic action of thyroglobulin is wholly dependent upon its organically contained iodine, and that artificially iodinated albumins and globulins other than the thyroid globulin do not possess this specific action. With these facts in mind—all of which strongly suggest a specific affinity of the thyroid cells *in vivo* for iodine—it seemed likely that certain of these manifestations could be produced *in vitro*, and if so, could be easily demonstrated. The thyroid seems still more favorable for such a demonstration when one recalls that seemingly more difficult functional characteristics of surviving cells of more complex organs, as for example the synthesis of hippuric acid by the kidney, and the production of glycogen from dextrose and of urea from ammonium carbonate by the liver cells, have been demonstrated experimentally.

The method of perfusion was chosen because of its simplicity and because similar operations can be carried out *in vivo*. In all these experiments the thyroids, kidneys and spleen of dogs were used. Up to the present 33 experiments have been made.

No anatomically normal glands were used, primarily because of the difficulty of obtaining them in this region, secondly because

of the technical difficulty of preparing and perfusing so small a structure, and lastly because colloid glands (goitres) have all the physiological characteristics of normal glands and are readily obtained. For studies in thyroid function one needs only physiologically active hyperplasias and the physiologically normal glands free from degenerative changes, of which hemorrhage and cyst-formation are the most common.

The perfusion apparatus used is represented in the accompanying diagram (fig. 1). It was arranged so that it could be sterilized in an ordinary autoclave after being set up for an experiment. A Luer syringe of 10 cc. capacity, with an especially wide nozzle, was used as a pump. Power was obtained from a motor so geared that the number of pump strokes per minute could be adjusted to any rate between 18 and 60. The length of stroke (volume of fluid pumped) was also adjustable from 0.1 to 5 cc.

A mercury manometer was connected with the arterial system, and also served as an elastic cushion, since as little rubber as possible was used in the various connections. In the preliminary experiments 65 cc. of Ringer's solution plus 10 cc. of erythrocytes was used, while in all of the experiments here reported 50 cc. of defibrinated blood and 25 cc. of Ringer's solution were used. This was for convenience only. The circulatory system from the reservoir to the organ box held 25 cc., and as a practical measure it was found best to use Ringer's in getting out the air and in testing the vessels for clots before the blood was introduced into the reservoir.

Oxygen was introduced into the reservoir at first from above, and allowed to rise through a column of glass beads, through which the venous blood also had to pass in the opposite direction. This method of introducing oxygen was sufficient to supply the oxygen needs of the thyroid, but it was found quite inadequate for organs requiring large amounts of oxygen, like the kidney and spleen. Introducing the oxygen from below and allowing it to bubble through the column of blood in the reservoir relieved this difficulty. Frothing was controlled by a perforated porcelain disc placed in the reservoir well above the level of the blood,

and onto this disc the venous blood from the organ was allowed to fall.

The blood flow was kept constant for all thyroid lobes over 15 grams in weight and for all kidneys and spleens—approximately 8 cc. per minute. Three difficulties common to perfusions may be mentioned. First is the occasional occurrence of

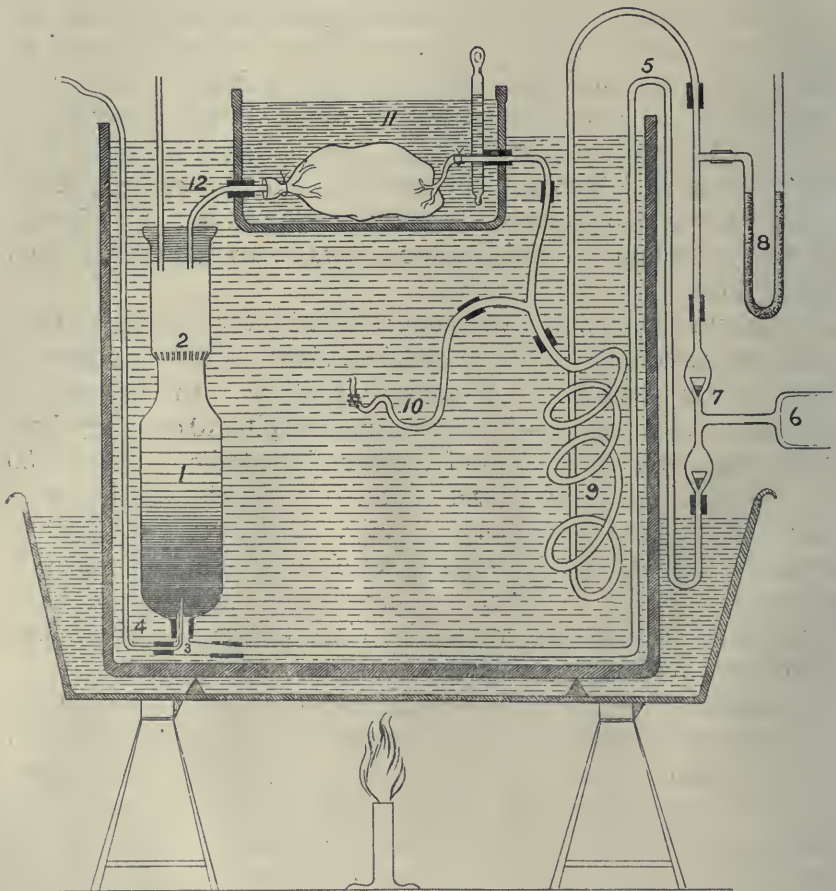


FIG. 1

1, Reservoir; 2, perforated disc; 3, outlet for blood; 4, oxygen tube entering through outlet; 5, tubing to pump; 6, pump; 7, valves; 8, manometer; 9, warming coil; 10, shunt tube; 11, organ box; 12, venous backflow to reservoir.

clots in the veins or arteries. The second difficulty is in carefully dissecting and ligating all vessels save the superior thyroid artery and all veins other than those joining the internal jugular from the thyroid. We were surprised, however, to find it easier to obtain thyroid preparations free from leaks and with adequate veins (the thyroid is singular among the organs in the number and great size of its veins) than splenic or renal perfusions. Anomalous arrangements of the thyroid veins occasionally render a lobe unsuitable for perfusion. The same trouble occasionally arises in the case of the spleen and kidney.

Thirdly, oedema in varying degrees is present in all cases. This is most marked with the kidney and spleen and least with the thyroid. Good thyroid preparations perfuse readily at 20 to 50 mm. Hg. pressure after the initial serum constriction of the arteries, which usually passes off in from 6 to 10 minutes. During this period the pressure may rise to 85 or 100 mm. Hg. With the low pressures one may perfuse the thyroid from 2 to 3 hours with less than 10 per cent increase in weight. With the spleen and kidney the necessary pressure is always much higher, although both show the usual transient serum constriction of the vessels. In our experiments the average pressure for the kidney was 80 to 100 mm., while with the spleen it was 70 to 90 mm. The richer and freer lymphatic drainage of the thyroid, together with the short wide capillary system and consequent short circulation time, compensates for the increased permeability of the vessels of surviving organs. Likewise the kidney with its very long and narrow capillary system and the spleen with its relatively long, peculiar and ill understood capillary system, together with the fact that all lymphatics are necessarily ligated, offer little or no compensation against the endothelial injury. Upon the whole, the goitrous thyroid probably gives the easiest and most satisfactory perfusion of all the body glands.

In young dogs with actively hyperplastic glands, it is usual to obtain perfusions free from signs of leaks even after two hours. With colloid glands or glands with thickened capsules, slight capillary extravasations of blood into the surrounding Ringer's solution are frequently seen. With the kidney and spleen, even

though using greater care than with the thyroid, we have never been able to prepare them so that no escape of blood took place during the perfusions. Doubtless the difference in pressure necessary for thyroid perfusions, on the one hand, and for kidney and spleen, on the other, account in part for this difficulty.

Histological examinations were made both of the perfused and of the control organs, and the groups have been made as in previous work on the basis of the histological condition. For convenience, four arbitrary thyroid groups have been made, viz.: (1) marked hyperplasia; (2) moderate hyperplasia; (3) early hyperplasia; and (4) colloid glands. The iodine determinations have been brought into relationship with these groups.

Histological examinations have proved a reliable additional check on the physiological condition of the gland cells. Hyperplastic glands often undergo autolysis within an hour or two after removal from the body. This is characterized by desquamation of the follicular epithelium, and, as will be seen later, is associated with a loss of thyroid iodine rather than a gain in iodine during perfusion.

The thyroids, spleens and kidneys were carefully dissected and placed in Ringer's solution in the ice box. Perfusions were usually conducted for about one hour—the shortest being 38 minutes and the longest 5 hours and 30 minutes. Potassium iodide was the only form of iodine used. This salt was used in amounts varying from 5 to 32 mgm. in a total perfusate of 75 cc. Following the perfusion the gland was washed with warmed Ringer's until the fluid came away clear. This usually required from 500 to 1000 cc., depending somewhat on the size of the gland and its histological condition. The same technique was carried out with the kidney and spleen perfusions.

The following protocol is introduced to illustrate the method and data obtained.

Experiment 11, November 5, 1914. Bull-terrier; female; weight, 14.6 kg.

9.50. Etherized, bled, defibrinated.

10.30. Right lobe of thyroid removed; weight 16.5 grams. In Ringer's in ice box.

10.45. Spleen removed; weight, 116.0 grams. In Ringer's in ice box.

11.00. Left kidney removed; weight, 66.0 grams. In Ringer's in ice box.

Left thyroid removed; weight, 16.0 grams. In Ringer's in ice box.

11.20. Perfusion of right thyroid started. 50 cc. defibrinated blood + 25 cc. Ringer's + 5 mgm. KI.

11.23. Pulse, 22; drops per minute, 134; pressure, 72.5; temperature, 35.

11.30. Pulse, 22; drops per minute, 134; pressure, 55; temperature, 36.

12.00. Pulse, 22; drops per minute, 135; pressure, 50; temperature, 37.

12.20. Perfusion stopped. Weight of lobe = 20 grams. Washed with 570 cc. Ringer's. Spec. for iodine and histology; gland, organ box fluid and total fluids.

Left thyroid not perfused.

2.10. Perfusion of left kidney using 25 cc. Ringer's + 50 cc. defibrinated blood + 5 mgm. KI.

2.15. Pressure = 92 mm.; pulse, 22; drops, 106.

2.25. Pressure = 85 mm.; pulse, 22; drops, 110; temperature 36.

3.00. Pressure = 85 mm.; pulse, 22; drops, 110.

3.10. Perfusion stopped. Weight of kidney 98 gm. Washed with Ringer's. Spec. for iodine and histology; gland, organ box fluid and total fluids.

4.05. Perfusion of spleen using 25 cc. Ringer's + 50 cc. defibrinated blood + 5 mgm. KI.

4.07. Pulse, 22; drops, 78; pressure, 90; temperature, 34.5.

4.20. Pulse, 22; drops, 89; pressure, 76.

5.05. Perfusion stopped. Weight of spleen, 162 grams. Washed and spec. for iodine and histology.

Histology. Thyroid, colloid—early hyperplasia. Structure well preserved. Kidney, oedematous. Spleen, highly oedematous.

Iodine. Right thyroid (perfused) = 1.38 mgm. per gram. dried.

Left thyroid (not perfused) = 0.54 mgm. per gram dried.

Kidney (not perfused) = 0.00 mgm. per gram dried.

Spleen (perfused) = 0.03 mgm. per gram dried.

This is the average protocol and shows the great difference between the thyroid, on one hand, and the spleen and kidney,

on the other, as regards the amount of KI absorbed when exposed to the same amounts for the same interval of time. These data for all the experiments of this type are collected in the following table.

TABLE I

(a) Thyroid perfusions									
HISTOLOGICAL CONDITION	NO. CF PERFU- SIONS	IODIN CONTENT PER GM. IN MGM. CONTROL LOBE			IODIN CONTENT PER GM. IN MGM. PERFUSED LOBE			AVERAGE IN- CREASE PER GM. IN MGM.	PERCENTAGE INCREASE
Marked hyper- plasias.	6	E.	M.	Av.	E.	M.	Av.	0.52	743
		0.00			0.22				
		0.09	0.08	0.07	1.31	0.85	0.59		
Moderate hyper- plasias.	4	0.01			0.11			0.51	340
		0.38	0.10	0.15	1.46	0.54	0.66		
Colloid—early hy- perplasias.	3	0.33			0.73			0.67	143
		0.55	0.54	0.47		1.11	1.14		
				1.38					
Colloid glands.	3	0.77			0.86			0.19	18
		1.54	0.78	1.03	1.85	0.94	1.22		
(b) Kidney perfusions									
	17	0.00			0.00				
					0.09	0.01	0.03	0.03	
(c) Spleen perfusions									
	8				0.00				
		0.00				0.03	0.03	0.03	
					0.08				

In the above table the thyroid experiments have been grouped according to their histological structure, and for comparison the results of the kidney and spleen perfusions have been added.

It will be noted that the thyroid takes up KI very rapidly, while the spleen and kidney do so, if at all, only to a slight

degree. We are inclined to think the presence of iodine in any specimen of kidney or spleen indicates either incomplete washing or death of the cells. The iodine taken up by the thyroid cannot be washed out by prolonged washing provided the gland is surviving. The average amount of KI taken up is nearly the same in the three degrees of active hyperplasia, while the percentage increase obviously varies with the degree of hyperplasia. The colloid glands or those nearly saturated with iodine take up very little. No completely saturated glands were used. There is a suggestion that the ability of the gland to absorb KI varies with the degree of hyperplasia and inversely with the iodine content. The differences are too slight to indicate any biological significance or even that the whole process may not be physical. On careful analysis, however, these experiments suggest that other factors are intimately concerned, and it has seemed best to present this analysis under the following groups:

1. Relation of other organs to the absorption of KI.
2. Relation of dying and surviving thyroid cells to the absorption of KI.
3. Effect of varying the concentration of KI.
4. Effect of KCN on the power of the thyroid cells to take up KI.
5. Attempt to wash out the iodine in glands in which the iodine content has been raised by the administration of iodine for a period of two weeks.
6. Effect of *in vivo* perfusions.
7. Is the iodine of either the *in vivo* or *in vitro* perfusions pharmacologically active?

1. *Effect of perfusion of other organs.* Only the kidney and spleen have been used. Normally in these organs one never finds a trace of iodine. As shown in Table I above, the kidney and spleen after perfusion contain only traces, the extremes for the kidney being 0.00 and 0.09 mgm., with a mean of 0.01 mgm. and an average of 0.03. The spleen perfusions were similar. As nearly one half showed no detectable iodine after washing, and in three instances unwashed spleens and kidneys showed a minimum of 0.03 mgm. and a maximum of 0.08, we are inclined

to consider the small amounts retained as extracellular—either in the vessels, lymphatics or renal tubules. Certainly one can state that the kidney and spleen effects are in no way similar to those of the thyroid.

2. *Relation of dying and surviving thyroid cells to the absorption of KI.* As indices of dying and surviving cells we have used the oxygen consumption and the histological condition of the glands. Both give valuable information, and, so far as our observations have gone, only those glands which were histologically intact maintain their oxygen consuming capacity. All the oxygen determinations were made with the old Barcroft method (5) and were comparisons of the arterial and venous bloods. The thyroid consumes very little oxygen per gram as compared with the spleen, and the kidney has a very high oxygen consumption, as noted by Barcroft.

It was found that the actively hyperplastic glands consumed more oxygen than the colloid glands. This was anticipated and is additional physiological proof that the hyperplastic gland is physiologically more active than the colloid gland. The fact, therefore, that the enormous blood supply of the thyroid has little to do with the oxygen needs of the gland is a matter of the greatest interest and is a point worthy of studying, since the thyroid has so many features in common with the lung—embryological, anatomical and physiological.

The most prominent histological feature of thyroid death is the desquamation of the alveolar epithelium. This histological change is given great prominence in most studies in pathological anatomy, and many speculations as to its significance (e. g., trauma, toxicity, hyperactivity, etc.) have been offered. Our experience leads us to reject all explanations other than that it is an index of cell death and autolysis. Under favorable conditions of asphyxia and temperature, epithelial desquamation may set in an hour after removal from the living dog.

The relation of KI absorption to cell death is shown in the following table.

In those cases with marked autolysis there was always a loss of iodine, although in all cases at least 5 mgm. and in one case

TABLE II

(a) *Thyroid perfusions with marked autolysis*

HISTOLOGICAL CONDITION	NO. OF PERFU- SIONS	IODIN CONTENT PER GM. IN MGM. CONTROL LOBE			IODIN CONTENT PER GM. IN MGM. PERFUSED LOBE			AVERAGE DE- CREASE OR IN- CREASE PER GM. IN MGM.	PERCENTAGE DE- CREASE OR IN- CREASE
		E.	M.	Av.	E.	M.	Av.		
Marked hyper- plasias.....	2	E. 0.00	M.	Av. 0.10	E. 0.00	M.	Av. 0.07	-0.03	-30
		0.20			0.14				
Colloid—early hy- perplasia.....	1			0.38			0.25	-0.13	-34
Colloid glands.....	1			0.98			0.54	0.44	-45

(b) *Thyroid perfusions with slight autolysis*

Marked hyper- plasia.....	1			0.08			0.22	+0.14	+175
Colloid—early hy- perplasia.....	1			0.25			0.40	+0.15	+60

20 mgm. KI were added to the perfusate. In the two cases with mild epithelial desquamation, there was a slight gain recorded, but only about one-fourth that observed in the corresponding grades of hyperplasia with intact glands. It seems certain from these observations that the dying thyroid cells no longer exhibit the power of taking up and holding KI, or, in other words, our experiments indicate that the phenomena of absorption and retention of KI are characteristics of the living cells as judged by the O_2 consumption and the histological structure.

3. *Effect of varying the concentration of KI in the perfusate.* The data are given in the following table arranged according to anatomical structure and KI concentration.

From these figures it would appear that the absorption of KI is independent of the concentration. The lowest amount used was 5 mgm. and the highest 32 mgm. in a constant quantity of

TABLE III

(a) <i>Marked hyperplasia</i>					
EXP. NO.	IODIN CONTENT PER GM. IN MGM. CONTROL LOBE	IODIN CONTENT PER GM. IN MGM. PERFUSED LOBE	AMOUNT KI USED IN MGM.	INCREASE IN IODIN	PERCENTAGE INCREASE IN IODIN
10.....	0.08	0.97	5.0	0.89	1100+
29.....	0.09	0.62	10.0	0.53	580+
22.....	0.08	0.22	10.0	0.14	175+
8.....	0.08	0.85	12.0	0.77	960+
5.....	0.00	1.31	17.0	1.31	
33.....	0.08	0.54	20.0	0.46	575+
(b) <i>Moderate hyperplasia</i>					
27.....	0.05	0.46	10.0	0.41	820+
7.....	0.38	1.46	15.0	1.08	280+
13.....	0.15	0.62	20.0	0.47	310+
4.....	0.01	0.11	32.0	0.10	1000+
(c) <i>Colloid-early hyperplasia</i>					
11.....	0.54	1.38	5.0	0.84	160-
12.....	0.33	0.93	10.0	0.60	180+
9.....	0.55	1.11	11.0	0.56	100+
(d) <i>Colloid glands</i>					
24.....	0.77	0.94	10.0	0.17	20+
26.....	1.54	1.85	10.0	0.31	20-
30.....	0.78	0.86	10.0	0.08	10+

perfusing fluid—75 cc. There was no histological evidence of autolysis in any of these experiments, and all glands were actively consuming oxygen. No explanation is offered for the wide differences in the amount of KI absorbed, which we believe are greater than could be accounted for on the basis of anatomical differences in the glands or of age and sex.

4. *Effect of KCN on the amount of KI absorbed.* Only two experiments have been made because of the necessity of having dogs with large accessory thyroids which could be utilized as controls for the lobe perfusions. A tabulation of these two experiments follows:

TABLE IV

THYROID LOBE	HISTOLOGICAL CONDITION	AMOUNT KI ADDED IN MGM.	AMOUNT KCN ADDED IN MG.M.	IODIN CONTENT PER GM. IN MG.M.	DURATION OF PERFUSION	PER CENT GAIN
Exp. No. 32						
Accessory lobe....	Marked hy- perplasia...			0.09		
Right lobe.....	Marked hy- perplasia...	10.0	100.00	0.11	1 hr. 3 min.	20
Left lobe.....	Marked hy- perplasia...	10.0	0.00	0.42	1 hr. 3 min.	360+
Exp. No. 33						
Accessory thyroid.	Marked hy- perplasia...			0.08		
Right lobe.....	Marked hy- perplasia...	20.0	50.0	0.08	1 hr.	0.0
Left lobe.....	Marked hy- perplasia...	20.0	0.0	0.54	1 hr.	575.0

In these experiments the effect of KCN in inhibiting the absorption of KI is striking. In each case the iodine content of the control and of the lobe treated with KCN are practically the same, while the lobes not treated with KCN gained 360 and 575 per cent respectively. In one experiment 10 mgm. KI was used in the perfusate of each lobe, while in the other 20 mgm. was used. The amounts of KCN used, 100 and 50 mgm., are doubtless far in excess of that necessary to induce the effect, and the result may therefore be merely that of dead cells. They are suggestive that KCN is able to inhibit the cell activity concerned in taking up KI, and it may be another example of the well known action of KCN in inhibiting cell activities in general. Up to the present no opportunity has offered of trying to wash out the KCN and to ascertain whether such glands are again capable of taking up KI—a fact well known in the case of developing eggs (1).

5. *Attempts to wash out the iodine of glands whose iodine contents had been raised by its oral administration for two weeks.*

It is known that iodine is excreted slowly from the thyroid normally, and under certain conditions (developing goitre) may disappear more rapidly. It therefore seemed plausible to at-

tempt to wash out some of it by perfusion, and that by using glands with high iodine contents one would be more likely to recognize its presence in the perfusate, as well as a decrease of iodine in the glands.

It is necessary to separate sharply those glands where the structure was preserved and those which after perfusion showed autolysis and desquamation of the alveolar epithelium, since, as stated above, all glands which showed well marked autolysis showed a loss of iodine whether perfused with or without the addition of KI.

The experiments in which at least one lobe showed preservation of histological structure are given in the following tabulation:

TABLE V
"Wash out" experiments

EXP. NO.	LOBE	WEIGHT IN GMS.	IODINE CONTENT, CONTROL LOBE, IN MG. PER GM.	IODINE CONTENT, PERFUSED LOBE, IN MG. PER GM.	TOTAL IODINE IN LOBE	DURATION OF PERFUSION	HISTOLOGICAL CONDITION	TOTAL IODINE IN MG.
21	Right.....	36.5		1.09	10.46	1 hr. 25 min.	Perfect preservation	0.30
	Left.....	28.0		0.85	5.32	1 hr. 15 min.	Moderate autolysis	5.12
23	Accessory Right.....	22.8	0.76	0.98	7.54	1 hr. 38 min.	Perfect preservation	0.52
	Left.....	25.2		0.54	2.99	1 hr. 38 min.	Marked autolysis	4.32
25	Right.....	19.5		1.54	7.70	1 hr. 2 min.	Perfect preservation	0.25
	Left.....	28.5		1.52	11.37	1 hr. 2 min.	Perfect preservation	
								0.71
28	Right.....	81.0	0.62		13.06	1 hr. 22 min.	Perfect preservation	Trace
	Left.....	84.0		0.69	15.87			

In all cases there was a loss of iodine—lowest in those whose histological structure was well preserved and highest in those showing the most marked autolysis. While such experiments are not conclusive, they suggest that even in surviving glands it is possible to wash out a small percentage of the total iodine, and that as death of the cells takes place, the loss is greatly increased. Under these experimental conditions the loss is entirely through the blood stream. If these experiments are in any sense comparable to what happens in life, it would indicate that the iodine is given off to the blood rather than to the lymph stream. Normally the excretion of iodine in one form and the taking up of iodine in another probably go on simultaneously, and both are under some physiological control. There are many reasons for supposing that this control is exercised through the blood stream directly (2).

6. *Are the results of in vitro perfusions similar to in vivo perfusions?* It is well known, and we have also many times mentioned the fact, that iodine is taken up by the thyroid *in vivo* with great rapidity from any form or mode of its administration thus far tested, but up to the present no attempt at what might be called an *in vivo* perfusion has been made.

The experiments were carried out as follows: One thyroid lobe was removed as a control. Both kidneys were ligated and 50 mgm. KI injected into a vein. After the proper interval of time the dogs were sacrificed and the remaining lobe dissected out and washed with Ringer's. Up to the present but two *in vivo* perfusions of one hour's duration (to compare with the *in vitro* perfusions) have been made.

They may be tabulated as in Table VI.

These results are approximately the same as regards the amount of KI taken up in 1 hour as those obtained with the *in vitro* experiments. The spleens and livers were not washed, and are therefore higher in iodine than the *in vitro* perfusions. These results bear out the many published reports of the distribution of iodine in animal tissues made 24, 48, 72, etc., hours after injection, viz.: that even in one hour the thyroid exhibits its striking selective activity for iodine. One may conclude, there-

TABLE VI

HISTOLOGICAL CONDITION OF LOBES	IODIN CONTENT CONTROL LOBE IN MGM. PER GM.	IODIN CONTENT PERFUSED LOBE IN MGM. PER GM.	IODIN CONTENT SPLEEN IN MGM.	IODIN CONTENT LIVER IN MGM.	TOTAL KI INTRODUCED INTO VEIN	DURATION OF PER-FUSION	TOTAL INCREASE IN IODINE IN MGM. PER GM.	PER-CENTAGE INCREASE
Moderate hyper-plasia..	0.32	0.77	0.06	0.03	50.0	1 hr.	0.45	140
Marked hyper-plasia..	0.12	0.48	0.06	0.03	50.0	1 hr.	0.36	300

fore that there is no difference between *in vivo* and *in vitro* perfusions of one hour's duration as regards the thyroid's affinity for KI, and these results by deduction add further evidence of the survival of the thyroid in the *in vitro* perfusions.

7. *Is the iodine deposited in the thyroid either by in vivo or by in vitro perfusions of one hour's duration pharmacologically active?*

This seemed to us a most important question. It is universally accepted that the activity of the thyroid depends on its iodine content, and we have many times demonstrated this by obtaining from the same animal several specimens of thyroid during a course of feeding iodine and found that, as the iodine content rose, the gland showed a corresponding rise in its pharmacological activity. So far as we have been able to ascertain, no tests of the pharmacological activity have been made with thyroid which has been exposed to iodine for less than four days, and in that time the iodine or at least a part of it has become active. In this report we will record our tests for the pharmacological activity of thyroid perfused for one hour both *in vivo* and *in vitro*, using the very sensitive test of Gudernatsch (3), viz., the effect on tadpoles.

The experiments were carried out as follows: Groups of 5 tadpoles were placed in agate-ware dishes and fed with 50 mgm. of the powdered thyroid every other day and fresh sheep liver was given for two-hour periods on alternate days. The perfused and control lobes of seven experiments, including the two *in vivo* per perfusions, were used. The experiments were begun

on May 18, 1915, and terminated on June 29. The water (tap water) was changed twice daily.

To our surprise no difference was noticed between the control and the perfused thyroids of the same animal whether *in vivo* or *in vitro* experiments. There were the usual differences in activity among the several experiments depending on their original iodine content as first noted by Lenhart (4). The iodine acquired by perfusion in one hour, whether *in vivo* or *in vitro*, is wholly inactive and the results are comparable to Lenhart's results with KI alone or KI added to thyroid or to artificially iodized proteins. It is the specific combination of iodine in the thyroglobulin (probably in the aromatic nucleus of an amino acid) which gives thyroid its specific pharmacological activity.

But as stated above, thyroids which have been exposed to KI *in vivo* for three to four days show a marked increase in activity proportional to the iodine increase. Clearly, then, we have evidence that the elaboration of this iodothyroglobulin requires a considerable interval of time, and also that its elaboration is probably a highly complex and specific chemical activity of the thyroid.

We do not know as yet whether the thyroid alone is capable of carrying out the complete reaction when given a salt of iodine, as KI, but it would seem that further work might not only answer this question, but indicate as well the length of time required for its elaboration as shown by definite increase in its specific activity on tadpoles.

In some work Dr. Graham has carried out in this Laboratory he noticed in a large series of human thyroids certain specimens whose pharmacological activity in tadpoles was less than it ought to have been on the basis of the iodine contents. At the time we had no explanation to offer, but in the light of the results with the perfused thyroids it seems probable that these human thyroid preparations contained iodine in excess of that specifically bound to the thyroid protein.

SUMMARY

Goitrous thyroids of dogs are perhaps the most easily perfused of all organs under conditions at all physiological. The method of perfusion was primarily utilized to ascertain whether salts of iodine were held in the surviving gland in quantities far greater than in other surviving tissues similarly treated, and if this was true, whether one could not partially involute actively hyperplastic glands *in vitro* as we know invariably happens *in vivo*—the changes in the living animal's thyroid being recognizable in from 36 to 48 hours. We have demonstrated the former, but the latter involves the grave difficulties of maintaining nutrition and of getting rid of products of metabolism. The technical and aseptic problems are readily overcome. We have little doubt that eventually it will be possible to partially involute an actively hyperplastic gland by some such method.

The question of the absorption of other salts than iodine as for example bromides, arsenic, etc., has not been investigated. It is well known that following the administration of bromides the thyroid retains a part temporarily, but it produces none of the effects or activities of iodine.

These experiments have also given an indication that the elaboration of iodothyroglobulin is a slow and probably complex process, and it is hoped that further study will lead to a definite conception of the minimum interval of time required for its production. Such knowledge for the iodine protein combination might be applicable to other protein compounds with inorganic substances whose chemical nature and function are little understood.

It was early recognized that the thyroid alone might not be able to transform KI into iodothyroglobulin. This also is a subject for investigation.

The fact that it is possible to wash out very small amounts of the stored iodothyroglobulin from surviving glands and very large amounts from dying glands is of interest in connection with the old controversy whether the thyroid secretion passes out through the lymphatics or blood vessels. With the technique we have used it was possible to separate the products of lym-

phatic drainage from those of the blood, because the thyroid was placed in a glass box filled with Ringer's solution and without any connection with the blood except for the accidental leaks. Several of these perfusions have gone for two hours without the escape of any blood into the organ box, although many torn lymphatic trunks opened directly into the box. In the wash-out experiments no iodine was detected in the "organ box fluid" in those glands free from leaks and surviving. This evidence favors the view that the iodothyroglobulin is given up directly to the blood stream.

CONCLUSIONS

1. Artificially perfused thyroids take up and retain KI to the same extent that *in vivo* perfused thyroids do.
2. This characteristic is not shared by the liver, kidney, spleen or muscle.
3. The amount of KI retained is independent of its concentration in the perfusion fluid.
4. Only surviving glands exhibit the ability of taking up KI.
5. KCN inhibits this activity of the thyroid.
6. It is possible to wash out with defibrinated blood a very small amount of the iodothyroglobulin in an hour's perfusion even in intact glands rich in iodothyroglobulin.
7. Autolyzing glands do not take up KI, and rapidly give up their stored iodine to the perfusate.
8. The KI stored in a thyroid gland from one hour's perfusion, whether *in vivo* or *in vitro*, is pharmacologically inactive.

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CONTRIBUTIONS TO THE PHYSIOLOGY OF THE STOMACH

XXVIII. FURTHER STUDIES ON THE ACTION OF BITTER TONICS ON THE SECRETION OF GASTRIC JUICE

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The present work was entered upon at the suggestion of Dr. Carlson for the purpose of determining whether the so-called stomachics or bitter tonics, acting in the mouth or in the stomach can affect first, the appetite, second, the quantity and quality of the gastric secretion, and in what way, in cases of cachexia. Concerning the action of these drugs on the hunger mechanism, we are not concerned in this paper. It has been shown (X1) for the normal man and dog that the so-called stomachics or bitter tonics acting in the stomach alone have no appreciable influence on the hunger mechanism when given in the usual therapeutic quantities. If the stomachics are taken by mouth in the ordinary way, all of them inhibit gastric tonus and hunger contractions in direct proportion to the intensity and duration of stimulation of the nerve endings in the mouth. What the above picture would be in a condition of cachexia we cannot say, but it is well to bear the above notation in mind when considering the results we have obtained with respect to appetite.

Carlson has found (X2), working with the normal man and the normal dog that the tonics acting either in the mouth or in the stomach, in therapeutic quantities have no effect, unless one of slight depression be considered, on the quantity or quality of the gastric secretion. His experiments were large in number and carried on over a long period of time. Previous to the above work, Borrisow (X3) has reported a series of twelve

sham feeding tests on one dog with gastric fistula and esophagotomy. Six of these tests were made after giving the dog tincture of gentian in the mouth. Throughout the series, the sham feeding was continued for one minute and the gastric juice was collected for two hours following the sham meal. In the case of the gentian tests, the sham feeding was instituted as soon as the profuse salivation induced by the bitters had ceased. In these latter tests the average quantity of gastric juice obtained was higher, while the acidity and pepsin concentration remained constant throughout the entire series. But as Carlson has pointed out (X2), it may be questioned whether a short series of tests on one animal can be held as conclusive, especially in view of the great individual variations (over 100 per cent) within each series.

It has been suggested that in the normal dog (and individual), the sensory nerves for the appetite sense in the mouth and stomach are in a state of maximum excitability so that no further increase in response to the bitter tonics is possible, and that the gastric secretion both quantitatively and qualitatively is optimum for the condition at hand. It is known that certain drugs do not elicit their specific action except in pathological conditions. Thus may be cited the antipyretics (X4) which in the normal animal (and individual) do not alter the temperature, but when this is abnormally high they cause a drop of greater or less extent. Hence may it not be that in order to determine the action of these bitter tonics on the appetite and gastric secretion we should make our tests under the pathological conditions in which they are being prescribed and have been prescribed for so many years in medicine, namely in conditions of cachexia? It was with this view in mind that the work described in the following pages was undertaken.

Cachexia is a condition of general emaciation and weakness, often associated with anemia and a wasting of the entire body. The condition is due to a diminished ingestion of food, a disturbed metabolism or an increased waste of the proteins and fats of the body. The practitioner deals with many species of cachexia. There is first, the cachexia due to chronic infection,

the septic, the cancerous and the syphilitic cachexia; second, the cachexia due to alternations of the internal secretions, hypophyseopriva, strumipriva and suprarenal cachexia; third, the toxic cachexia due to chronic metal poisoning as saturnine, mercurial and arsenical cachexia; the cachexia due to improper food as scurvy and pellagra (?); and lastly, the cachexia due to hemorrhage or wasting discharges. No matter what the cause of the cachexia is, and we have seen how varied it may be, it has been the wont of them who minister to the ills of humanity, from the primitive medicine man, whose records we find in the earliest history, to the skilled practitioner of today, to endeavor to improve the appetite and gastric digestion of the patient, by the use of bitter tonics. That improvement has followed their employment, few practitioners doubt, but the question very naturally arises, how has this favorable influence been affected? Do the bitter tonics stimulate the appetite—or the gastric secretion—or both, or is their action purely psychical? Finally, do these bitters exert any action at all? Is it not possible, as Carlson has suggested (X2) that these tonics are given to convalescents who would continue to improve, tonic or no tonic—and the tonic, not the recuperative power of the patient gets the credit.

Hoppe (X5) reports that in no quantity do these drugs cause an increase in gastric secretion in the normal dog. Large amounts, however, cause a decrease in the quantity of the gastric secretion in the same animals. Continuing, he says, that in the sick dog, the use of one of the drugs is followed by an increase in the quantity and quality of the juice, that is, in the acidity and in the pepsin concentration. Large amounts of the drug cause no decrease in the secretion of gastric juice in the sick animal. This investigator gives us no figures nor tables of the results he obtained, neither does he in any way enlighten us as to the methods he pursued. It does not seem justifiable to accept as conclusive the statements in this report since we have no mention of the number of tests made, the method of procedure in the tests and the specific conditions of his animals, control tests, etc.

Before attempting to produce cachexia in our animals, a series of control experiments were conducted on the three then healthy, normal dogs, testing the action of the bitters first in the mouth, and then in the stomach, with respect to the amount of food eaten, and to the quantity and quality of the gastric secretion.

EXPERIMENTAL PROCEDURE

The three dogs employed were provided with accessory stomach pouches according to the Heidenhain-Pawlow method. In preparing the dogs we were careful to interfere as little as possible with the distribution of the vagi to the stomach pouch. When the bitters were given by mouth, they were soaked up in a small piece of cotton and the dog was compelled to chew upon this for one minute. When the tonics were tested for their action in the stomach, they were put into the main stomach directly by the stomach tube so as not to come in contact with the nerve endings in the mouth or oesophagus. The drugs were given never in excess of the therapeutic dose. Twenty-four hours were allowed to elapse between the tests. The gastric secretion was collected for the hour preceding feeding and for the hour succeeding the meal. The specimens collected were measured and examined separately for each hour. The drug was administered one hour before feeding and during this hour the dog was allowed to see and smell the food, hence in both cases we are dealing mainly with the appetite or psychic secretion. The food consisted of ground meat and bread and the dog was allowed to eat as much as it would. The amount eaten was recorded each day. The following bitters were used; tincture of gentian and calumba and elixir of iron quinin and strychnin. The pepsin concentration of the gastric juice was determined according to the Mett's method as used by Carlson (X6), and the result expressed in the average millimeters of digestion. The acidities were determined by titration with $\frac{N}{40}$ NaOH, and the percentages were expressed in terms of HCl as "free acidity" and "total acidity." In estimating the former, dimethyl-amido-azo-benzene was used as indicator, and phenolphthalein for the latter.

— Results on normal dogs

The results on the normal dogs with respect to appetite and the quantity and quality of the gastric secretion are summarized in tables 1 to 5 inclusive. The data in these tables indicate the following conclusions:

1. These bitters, acting either in the mouth or in the stomach, have no appreciable influence on the appetite.

2. These bitters, acting either in the mouth or in the stomach, have no consequential influence on the quantity of the gastric secretion, although a slight uniform depression is noted from their action in the stomach.

3. These bitters, acting either in the mouth or in the stomach, have no significant influence on the quality of the gastric secretion, although a slight rather uniform depression is observed in the free as well as in the total acidity from the action of the drugs in the mouth.

Hence I think that we may assert that the bitter tonics, acting either in the mouth or in the stomach of the normal dog, have no influence on the appetite; and we may conclude with Carlson (X2) that these bitters acting in the mouth or in the stomach, have no effect on the secretion of gastric juice in the (normal) dog.

EXPERIMENTAL PROCEDURE ON CACHECTIC DOGS

After the above control tests had been conducted with the dogs in normal condition, we determined to induce the cachexia by producing a chronic anemia. For this purpose we bled our dogs large quantities daily—200 to 300 cc.—until between 800 and 1200 cc. had been taken from the dogs. Our purpose was to bleed the dogs so much and so constantly as to exhaust by over-strain, the power of the blood forming organs. The bleeding was accomplished by inserting a needle into the left ventricle of the heart and collecting the blood in vacuo. This procedure was highly dangerous as subsequent events showed. We lost one dog from acute anemia within an hour of its last bleeding. Within a few days, the animals recovered from the shock of

the hemorrhages and then the cachexia began to develop. At no time during the series of experiments, did the animals show a tendency to recover from their condition of cachexia. The emaciation was a constant process as a perusal of table 11 will indicate. The weight of the animals was recorded before attempts to produce cachexia were made, and on every third day thereafter. The animals became thin and listless, showed less interest in their food and surroundings; in a word, they were typically cachectic.

After the development of the cachexia, ten tests were made without tonics, and these were followed by an equal number of tests using the bitters first in the mouth and later in the stomach. Another series was run in which the use of the tonics was frequently interspersed with controls. During the first hour periods, the dogs showed less interest in their food.

Results on cachectic dogs

Our results with respect to appetite and the quantity and quality of the gastric secretion of the cachectic dogs are summarized in tables 6 to 10 inclusive. The data in these tables indicate the following conclusions:

1. These bitters, acting both in the mouth and in the stomach, exert a favorable influence upon the appetite. The increase produced is not sufficient to bring the amount of food consumed up to normal, but the influence is definite and significant.

2. These bitters acting in the mouth cause an increase both in the quantity and quality of the gastric juice secreted during the hour following the meal. The qualitative increase is observed in both the free and total acidity, while the pepsin concentration remains fairly constant. The increase, it is true, is not great enough to bring either the quantity or the quality of the juice up to that secreted by the normal dog, but this increase is marked enough to be worthy of note.

3. These bitters, acting in the stomach, have no appreciable influence upon the quantity or the quality of the gastric secretion.

Comment on the results on cachectic dogs

From a consideration of the control experiments on the dogs in normal condition, we may feel assured that these animals possessed no idiosyncrasy for the drugs employed. With respect to normal dogs, the literature points to the conclusion that by themselves the bitter tonics are incapable of causing secretion of gastric juice either by acting in the mouth or in the stomach. Pawlow (X7) noted in dogs that the bitters acting in the mouth cause a copious flow of saliva, but leave the gastric gland perfectly quiescent; and not even when introduced into the stomach do they cause secretion of gastric juice. Our experiments seem to confirm the above, and also to indicate that the same picture holds true with respect to cachectic dogs, for in neither case, did we note an increase in the gastric secretion during the hour preceding the meal.

Do the bitter tonics augment the secretion of gastric juice indirectly by increasing the excitability of the nerve endings of taste in the mouth, and possibly the nerves of appetite sense in the stomach? This is the view emphasized by Pawlow (X7), but he does not adduce any experiments in its support. From the fact that the use of the drugs both in the mouth and in the stomach is followed by an increase in the amount of food consumed, one might conclude that the excitability of the nerve endings of taste in the mouth and the nerves of appetite sense in the stomach had been raised; but why, one may ask, does an increase in the gastric secretion follow in one case and not in the other? It is possible that the increase in the excitability of the gustatory nerve endings in the mouth renders the less palatable food more palatable, or mayhaps, it is the contrast between the taste of the bitter tonics and the food that does this, and thus causes the reflex mechanism for appetite secretion to work better.

Dr. Carlson suggested that possibly a conditional reflex had been established, that is, that the manipulation which the dog underwent in receiving the tonic became associated in its memory with the fact that feeding always followed this process, hence the psychic secretion might follow, tonic or no tonic, so long

as the "motions" of giving the bitters were gone through with. That memory associations may call forth a psychic secretion in the dog, is evidenced by the experiments of Pawlow in which he caused a gong to be struck at the same instant that feeding was instituted. After a few of the above tests, he found that the striking of the gong, without the appearance of the food was sufficient to call forth an appetite secretion from the dogs. For many reasons we do not believe that it was the condition of the reflex that called forth the increased secretion in our cachectic animals. First, the increased secretion did not commence during the first hour, but only after the food had been consumed: Second, the factor of manipulation was present as well when the bitters were put into the stomach by means of the stomach tube, and in this series, no increase in the gastric secretion was noted: Third, the increase in gastric secretion was observed the very first time that the bitters were used in the mouth of the cachectic dog, before an opportunity for the establishment of a condition of reflex had presented itself.

That the results obtained were not due to the convalescence of the animals, reference to the above tables, and to tables 12 and 13 especially, will indicate. The action of the bitters in the mouth seems definite. It is shown in table 12, that cessation of the use of the tonic during a series of its employment in the mouth causes a decrease both in appetite and in secretion, while restoration of the tonic is followed immediately by improvement in both of these conditions. Table 13, prepared at a much later date, indicates the effect of the tonic in the mouth when instituted during a series of no tonic tests.

Our results on the dog would seem to indicate that the use of the bitter tonics in cachexia is not without material foundation in medicine, but a final verdict as to their true efficacy cannot be given until other methods of investigation have been applied to them. We have yet to learn of their effect upon the time required for completion of gastric digestion, etc. The psychical effect, which has been eliminated in these experiments, may add to their beneficial value in the human. The mechanism by which our favorable results are obtained remains still an un-

solved problem, but as investigation proceeds step by step along the different lines, it is possible that the solution will become clearer, and that some day a complete answer may be given not only to the question "do the bitter tonics help in conditions of cachexia," but also to the question, "how do they aid in this condition?"

I desire to express my gratefulness to Professor Carlson for his guidance and help; to Dr. James J. Moorhead for his assistance in the preparation of the Pawlow pouches; and to Dr. Frank C. Becht for his aid in bleeding the dogs directly from the heart.

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 (X6) CARLSON: Am. Jour. of Phys., 1915, vol. xxxviii.
 (X7) PAWLOW: The work of the digestive glands, London, 1902, pp. 70-138.

TABLE 1
Appetite, normal dogs (food eaten in grams)

SERIES	NO. T	DOG 1			DOG 2			DOG 3		
		Avr.	Mxm.	Mim.	Avr.	Mxm.	Mnm.	Avr.	Mxm.	Mnm.
No tonics.....	10	325	540	225	280	370	240	280	340	250
Tonic in mouth..	10	282	400	200	259	370	160	280	300	250
Tonic in stomach.....	10	321	520	200	278	330	235	266	400	190

TABLE 2
Quantity of gastric secretion in cc., normal dogs

SERIES		NO. T	DOG 1			DOG 2			DOG 3		
			Avr.	Mxm.	Mnm.	Avr.	Mxm.	Mnm.	Avr.	Mxm.	Mnm.
Appetite secretion one hour	No tonics.....	10	2.9	4.6	0.1	3.17	14.8	0.1	1.22	4.2	0.2
	Tonic in mouth...	10	2.68	4.3	1.5	1.42	4.0	0.1	1.15	3.0	0.6
	Tonic in stomach..	10	1.53	4.0	0.1	2.31	4.3	0.1	1.17	3.8	0.1
First hour after feeding	No tonics.....	10	3.9	8.0	0.1	3.26	8.1	0.1	3.8	9.8	0.2
	Tonic in mouth...	10	3.6	16.8	0.1	4.39	31.0	0.2	2.30	7.0	0.2
	Tonic in stomach..	10	1.7	4.3	0.1	1.16	2.0	0.1	3.43	8.2	1.2

TABLE 3
Free acidity of gastric juice, normal dogs

SERIES		NO. T	DOG 1			DOG 2			DOG 3		
			Avr.	Mxm.	Mnm.	Avr.	Mxm.	Mnm.	Avr.	Mxm.	Mnm.
Appetite secretion one hour	No tonics.....	10	0.2380	0.3190	0.1459	0.1915	0.3737	0.0364	0.1780	0.3007	0.0638
	Ton. in mouth.....	10	0.1520	0.2462	0.0912	0.1755	0.2736	0.0364	0.0957	0.2918	0.0273
	Ton. in stomach....	10	0.2370	0.3098	0.1641	0.1960	0.3372	0.0638	0.1701	0.3007	0.0456
	No tonics.....	10	0.2188	0.2280	0.1732	0.3468	0.3828	0.2188	0.2680	0.3828	0.1276
First hour after feeding.....	Ton. in mouth.....	10	0.1117	0.1915	0.0273	0.3463	0.3828	0.3088	0.2279	0.3463	0.1276
	Ton. in stomach....	10	0.2128	0.2371	0.1915	0.1641	0.2736	0.0729	0.2695	0.3737	0.1459

TABLE 4
Total acidity of gastric juice, normal dogs

SERIES		NO. T	DOG 1			DOG 2			DOG 3		
			Avr.	Mxm.	Mnm.	Avr.	Mxm.	Mnm.	Avr.	Mxm.	Mnm.
Appetite secretion one hour	No tonics.....	10	0.3401	0.4010	0.1276	0.3228	0.4922	0.1003	0.2598	0.4010	0.1550
	Tonic in mouth...	10	0.1805	0.2462	0.0729	0.3214	0.5014	0.1732	0.2143	0.4010	0.1369
	Tonic in stomach.	10	0.3464	0.4102	0.2553	0.2340	0.4466	0.0729	0.2644	0.3646	0.1824
First hour after feeding	No tonics.....	10	0.3398	0.3737	0.1459	0.3799	0.5561	0.2280	0.3790	0.4831	0.2553
	Tonic in mouth...	10	0.2074	0.3007	0.1276	0.3828	0.6107	0.0638	0.3252	0.4384	0.2644
	Tonic in stomach..	10	0.3402	0.3737	0.3098	0.2857	0.3828	0.2371	0.3724	0.4922	0.2462

TABLE 5
Pepsin concentration in average mm. digestion, normal dogs

SERIES	NO. T	DOG 1			DOG 2			DOG 3			
		Avr.	Mxm.	Mnm.	Avr.	Mxm.	Mnm.	Avr.	Mxm.	Mnm.	
Appetite secretion on hour	No tonics.....	10	4.1	5.5	3.3	1.65	2.1	1.2	1.1	1.1	1.1
	Tonic in mouth...	10	4.2	5.5	3.25	1.6	2.1	1.1	1.1	1.1	1.0
	Tonic in stomach.	10	3.3	3.3	3.3	1.65	2.0	1.3	1.0	1.0	1.0
First hour after feeding	No tonics.....	10	3.5	3.8	3.4	2.4	2.7	2.2	2.1	2.9	1.4
	Tonic in mouth...	10	2.98	5.0	1.75	2.42	2.75	2.1	2.1	3.0	1.2
	Tonic in stomach.	10	3.6	4.0	2.8	2.0	2.0	2.0	1.75	2.5	1.3

TABLE 6
Appetite, cachectic dogs (food eaten in grams)

SERIES	NO. T	DOG 1			DOG 2		
		Avr.	Mxm.	Mnm.	Avr.	Mxm.	Mnm.
No tonics.....	10	62.8	180	0.0	148	280	0.0
Tonics in mouth....	10	154	280	35	186	340	0.0
Tonics in stomach...	10	163	310	0.0	241	350	102

TABLE 7

Quantity of gastric secretion in cc., cachectic dogs

S E R I E S		N O . T	D O G 1			D O G 2		
			A v r .	M x m .	M n m .	A v r .	M x m .	M n m .
Appetite secretion one hour	No tonics.....	10	0.50	2.0	0.0	1.15	5.0	0.0
	Tonics in mouth.	10	1.90	4.2	0.5	1.14	2.1	0.1
	Tonics in stomachs.....	10	1.06	3.1	0.0	1.14	2.2	0.0
First hour after feeding	No tonics.....	10	1.05	3.9	0.0	1.50	5.9	0.0
	Tonics in mouth.	10	2.50	5.0	0.2	3.15	7.7	0.1
	Tonics in stomach.....	10	1.20	3.5	0.2	1.80	2.5	0.1

TABLE 8

Free acidity of gastric juice, cachectic dogs

SERIES		NO. T	DOG 1			DOG 2		
			Avr.	Mxm.	Mnm.	Avr.	Mxm.	Mnm.
Appetite secretion one hour	No tonics.....	10	0.1640	0.3281	0.0	0.1585	0.3007	0.0820
	Tonics in mouth.	10	0.1784	0.3828	0.0	0.0516	0.0912	0.0
	Tonics in stomach.....	10	0.1868	0.3281	0.0456	0.1124	0.1915	0.0638
First hour after feeding	No tonics.....	10	0.2051	0.2918	0.1185	0.2042	0.3463	0.0729
	Tonics in mouth.	10	0.2309	0.4375	0.0912	0.2383	0.3281	0.1003
	Tonics in stomach.....	10	0.2006	0.3098	0.1550	0.2371	0.2918	0.0182

TABLE 9

Total acidity of gastric juice, cachectic dogs

S E R I E S		N O . T	D O G 1			D O G 2		
			A v r .	M x m .	M n m .	A v r .	M x m .	M n m .
Appetite secretion one hour	No tonics.....	10	0.2780	0.4010	0.1550	0.2644	0.3919	0.2006
	Tonics in mouth.	10	0.2995	0.4649	0.1276	0.1763	0.2280	0.0912
	Tonics in stomach.....	10	0.2431	0.4466	0.0912	0.2614	0.2918	0.2006
First hour after feeding	No tonics.....	10	0.2871	0.3646	0.2097	0.2643	0.4375	0.1003
	Tonics in mouth.	10	0.3562	0.5787	0.1732	0.3268	0.4193	0.2006
	Tonics in stomach.....	10	0.2871	0.4102	0.1185	0.3068	0.4284	0.1885

TABLE 10

Pepsin concentration in average mm. digestion, cachectic dogs

SERIES		NO. T	DOG 1			DOG 2		
			Avr.	Mxm.	Mnm.	Avr.	Mxm.	Mnm.
Appetite secretion one hour	No tonics.....	10	Not	suff.	juice	0.25	0.25	0.25
	Tonics in mouth.	10	1.35	1.7	1.0	Not	suff.	juice
	Tonics in stomach.....	10	0.9	0.9	0.9	1.4	1.4	1.4
	No tonics.....	10	1.7	1.7	1.7	0.85	1.1	0.7
First hour after feeding	Tonics in mouth.	10	1.85	1.9	1.8	1.5	1.8	1.1
	Tonics in stomach.....	10	1.35	1.7	1.0	0.8	0.8	0.8

TABLE 11

Table of weights of dogs 1 and 2 during cachexia

DATE	DOG 1	DOG 2	DATE	DOG 1	DOG 2
	<i>kilo</i>	<i>kilo</i>		<i>kilo</i>	<i>kilo</i>
May 6, 1915	10	9.2	May 25, 1915	7.9	7.6
Cachexia induced;	weights	following	May 27, 1915	7.4	7.3
May 11, 1915	8.6	8.7	May 31, 1915	7.4	7.3
May 14, 1915	8.4	8.0	June 8, 1915	7.1	7.3
May 16, 1915	8.2	8.0	June 15, 1915	7.1	7.3
May 18, 1915	7.9	7.6	June 21, 1915	7.1	7.4
May 21, 1915	7.9	7.6			

TABLE 12

Detail of one series of experiments on cachectic dog No. 1. Omitting the tonic from the mouth one day (No. 39)

Experiment No.....	36	37	38	39	40	41	42
Date.....	5/17/15	5/18/15	5/19/15	5/20/15	5/21/15	5/22/15	5/23/15
Drug used.....	Tincture gentian	Tincture gentian	Tincture gentian	None	Tincture gentian	Tincture gentian	Tincture gentian
Appetite secretion one hour	Quantity..	1.6 cc.	0.5 cc.	1.4 cc.	None	3.2 cc.	1.0 cc.
	Free acid..	0.1459			0.2462	0.1459	0.2736
	Total acid..	0.2736		0.1276	0.3372	0.3646	0.4010
	Pepsin.....				1.7		1.0
Amount food eaten in grams	110	35	280	180	310	45	190
First hour after feeding.....	Quantity..	5.0	1.85	0.2	None	2.0	4.3
	Free acid..	0.0912	0.1094		0.2371	0.2736	0.2371
	Total acid..	0.1732	0.1915		0.3554	0.4556	0.3828
	Pepsin.....	1.8			1.8	1.9	

TABLE 13

Detail of one series of experiments on cachectic dog No. 1. Giving tonic in the mouth one day, (No. 56)

Experiment No.....	53	54	55	56	57
Date.....	6/7/15	6/8/15	6/9/15	6/10/15	6/11/15
Drug used.....	none	none	none	Tincture gentian	none
Appetite {					
secretion {	Quantity.....	0.3 cc.	0.8 cc.	0.9 cc.	0.5 cc.
one hour {	Free acid.....				0.3 cc.
	Total acid.....				
	Pepsin.....				
Amount food eaten in grams..	260	250	160	390	210
First hour {	Quantity.....	1.9	0.6	0.5	3.6
after {	Free acid.....	0.2280			0.3828
feeding {	Total acid.....	0.3281			0.5196
	Pepsin.....				1.83

THE CENTRAL ACTION OF DIGITALIS AS TESTED BY THE CARDIO-INHIBITORY CENTER

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Traube¹ in 1872 came to the conclusion that preparations of digitalis stimulated the cardio-inhibitory center of the medulla, basing his deduction on the effect of sectioning of the vagus trunk after digitalis. Later Cushny² 1897 reached a similar conclusion in his study of the heart during the systemic action of digitalis. Certain cardiac phenomena lead him to the further conclusion that digitalis paralyzed the vagus center in its late action. Lewis³ 1909 and Mackenzie⁴ 1910 presented evidence suggesting that digitalis produces a change in the conductivity of the heart, a change similar to that upon vagus stimulation.

We have attempted to more definitely analyze the action of digitalis on the nervous center of the cardiac-inhibitory pathway by the study of the effects of digitalis when perfused through the isolated head and brain.

METHODS

The turtle is an animal that lends itself readily to this analysis, since the cardio-inhibitory nervous mechanism is well developed and the augmentary apparatus poorly developed, if not in fact

¹ Traube: *Gesammelte Beiträge zur Pathologie und Physiologie*. 1871, vol. i, p. 190 and 1872, vol. ii, p. 907.

² Cushny: *Action of the Digitalis Series on the Circulation*. *Jour. of Exp. Med.*, 1897, vol. ii, p. 233.

³ Lewis: *Auricular Fibrillation and Relationship to Clinical Irregularity of the Heart*; *Heart*, 1909, vol. i, p. 306.

⁴ Mackenzie: *Digitalis*; *Heart*, 1910, vol. ii, 273.

absent. Our method has been to completely isolate the head from the general circulation and to perfuse the head and brain. We ligate one carotid artery and insert a cannula into the cephalic end of the other. The jugular veins are also ligated and the cervical cord severed. In one jugular vein we have inserted an out-flow cannula. The vagus nerves have been maintained intact, but all other tissues severed in the neck region. This preparation maintains only one connection between the head and the body, namely, that through the vagus trunks. All vascular pathways from the cranial vessels to the systemic vessels are closed, and all nervous pathways likewise eliminated. If solutions perfused through this preparation stimulate the vagus

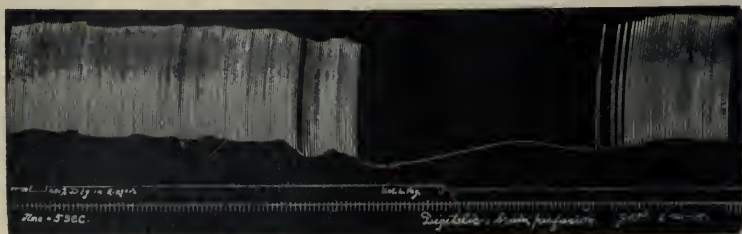


FIG. 1. Perfusion of digitalis through the cephalic end of the carotid of the isolated head. The vagus nerves only were in connection with the rest of the body. The strength of digitalis used was 0.01 per cent in Ringer's solution. Total inhibition occurred. At the mark the left vagus was cut but inhibition continued.

nucleus in the medulla that stimulation will be accompanied by changes in the heart. We have concentrated attention on this one test. During perfusion we record the ventricular rhythm and amplitude by direct attachment of the ventricular apex to the recording lever.

Soluble digitalis (Mallinckrodt) has been used though supplementary experiments have been performed with strophanthin. The solutions were made up in Ringer's solution (0.7 per cent sodium chloride + 0.03 per cent potassium chloride + 0.026 per cent calcium chloride).

EXPERIMENTAL RESULTS

When the preparation is ready, Ringer's solution is perfused through the brain and the rate of out-flow of fluid noted, though not recorded. After the normal rhythm was recorded, digitalis was substituted for the Ringer's solution. Owing to the marked arterial constriction induced by digitalis, we have found it necessary to use relatively higher pressures, namely, 20 to 46 cm. of

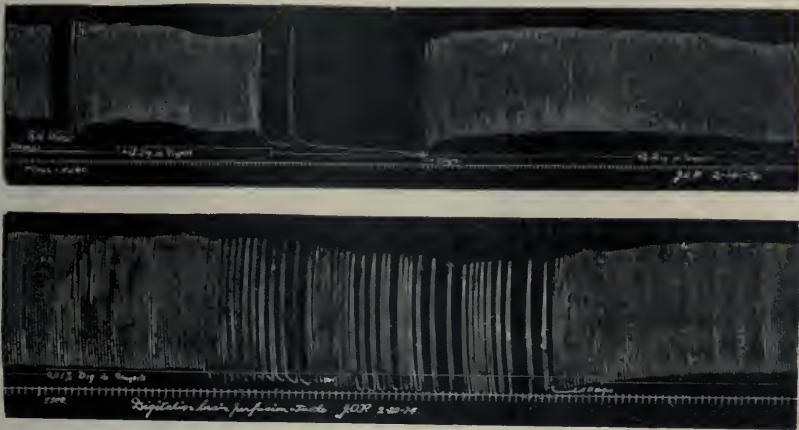


FIG. 2. Brain perfusion of 0.1 per cent digitalis in Ringer's solution. When cardiac inhibition occurred the digitalis was removed, and after three minutes the right vagus was cut. The heart immediately began its normal rhythm. A second perfusion of digitalis produced no change in the heart.

FIG. 3. Second perfusion of the brain with 0.1 per cent digitalis in Ringer's solution. For the effects of the first perfusion see figure 4. The left vagus had been sectioned in the first test, the right kept intact. Slowed but irregular rhythm characterizes this record. At the point marked and while the digitalis was still perfusing the right vagus was sectioned. The heart at once resumed its normal rhythm.

water. Solutions of different concentrations of digitalis were used, but chiefly, 0.01 per cent and 0.1 per cent. The brain reaction occurs after a somewhat long latent period. The latent period was relatively shorter in the stronger solutions. The different concentrations produce qualitatively the same results. See figures 1 and 2.

Influence on the rate. Perfusion of 0.01 per cent digitalis through the brain produces a slowing or complete inhibition of the heart. In these preparations of the turtle the latent period is relatively long, from 20 seconds to 5 or more minutes. When the drug begins to act, the heart rate suddenly and sharply decreases to a slower rate, and after 2 or 3 beats stops abruptly in complete inhibition, see figures 1 and 2. During the beats at a slowed rhythm the ventricle usually but not always exhibits a greater amplitude, i.e., compensatory. In continued perfusion of the drug of this strength the inhibition is as a rule very much prolonged, but recovery occurs on removal of the drug, figure 4. With the stronger concentrations the rhythm is slowly but gradually resumed and may escape to the original normal rhythm even during perfusion. If the drug is used for short intervals only then the rhythm is more gradually resumed though there may be periods of further slowing at irregular intervals. The explanation of the recurrent periods of slowed rhythm after digitalis perfusion we attribute to the prolonged and cumulative action of the drug. This has appeared over and over in the series of experiments. The recovery of the rhythm during continued perfusion of digitalis, especially with the stronger solutions, is attributed to toxic paralysis of the vagus center, a confirmation of Cushny's determination by other methods. The escape during perfusion can not be ascribed to fatigue of the vagus trunk or of the nerve endings, since direct electrical stimulation of the vagus is effective during this time. The long latent period was unexpected, and it is suggested that it is bound up with the slow diffusion of digitalis from the capillaries into the brain tissues. The marked constriction of the arterioles in itself blockades the flow of solutions through the vessels. Stopping the flow of Ringer's in the normal preparation does not lead to arrest of the rhythm of the heart though it favors arrest after digitalis has once been used.

Influence on conductivity. When the heart is slowed under the influence of digitalis through the medulla, it is often noted that a degree of heart block occurs. In other words the sinus and the ventricle contracted in a 2-1 or 3-1 rhythm. Sometimes the

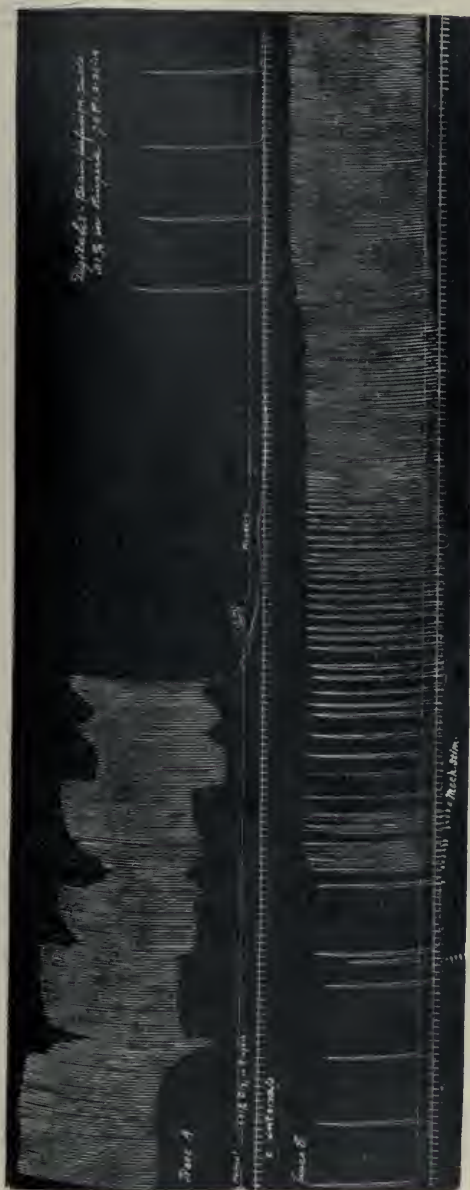


FIG. 4. Perfusion of the brain with 0.01 per cent digitalis in Ringer's solution, first test. Tracings A and B form one continuous experiment. The cutting of the left vagus had no effect on the total inhibition. Mechanical stimuli produced contractions during early recovery. The irregularities in the early part of the tracing are due to uncontrolled skeletal muscle contractions of the cervical region, i.e., digitalis effects on the cervical cord.

ventricle was held in complete pause while the sinus gave a definite rhythm. This is undoubtedly a case of reduced conductivity, a characteristic phenomenon under vagus stimulation.

This phenomenon of reduced conductivity can not of necessity be upon any stimulating action on the vagus endings as explained by Mackenzie. In this preparation no digitalis comes in contact with the heart, hence none with the vagus endings. We must ascribe the effect to the central stimulation.

Sectioning of the vagi during the inhibitory pause from brain perfusion. The literature contains numerous statements of the unequal influence of the right and left vagus nerves, not only in turtles but in mammals as well. Garry⁵ 1911 has given a fuller analysis of this variation in the turtle. He showed that the rhythm of the right vena cava controls the rhythm and sequence of the heart, though the left vena cava is also rhythmic. He found that the right vagus was in closer relation to the rhythmic control of the heart than the left, and that the left has greater control over the conductivity. He ascribes the pause following the stimulation of the left vagus as due to blocking produced by the decreased conductivity rather than to a direct inhibition of the rhythm.

During the pause from central stimulation by digitalis we have tested the relative efficiency of the two nerves by alternate sectioning. If the left vagus is sectioned no change occurs in the rhythm or state of inhibition. But after the right is sectioned the heart always resumes its normal rhythm. Sectioning of the vagus nerves is the final proof that the results presented here are of central origin, a proof that is more complete under the conditions of our experiments than under the conditions which exist in systemic application of digitalis.

SUMMARY

1. Digitalis strongly and directly stimulates the cardio-inhibitory center of the medulla.

⁵ Garrey: Rhythm in the Turtle's Heart in Comparison of Action of the Vagi; Amer. Jour. of Physiol., 1911, vol. xxviii, p. 330.

2. Paralysis of the center by excessive action is shown by the escape of the heart during perfusion and by the failure of subsequent perfusions to produce inhibition.

3. Cumulative and persistent action on the inhibitory nerve center is indicated by the recurrence of various degrees of inhibition during subsequent perfusion with Ringer's solution.

4. Not only is the rhythm inhibited but there is a blocking of the passage of the contraction wave from the veins and sinus to the ventricle by a decrease in conductivity.

TABLE I
Showing the effect of digitalis upon the cardio-inhibitory center of the medulla
 The circulation of the brain was isolated and the effect of brain perfusion was judged by the vagus control of the heart action

DATE	DIGI- TALIS	TIME OF PERFUSION		NORMAL HEART RATE PER MINUTE		NORMAL AMPLI- TITUDE		DIGITALIS EFFECTS ON THE HEART		RECOVERY LEVEL AFTER DIGITALIS		REMARKS
		min.	sec.	mm.	mm.	rate	ampli- tude	rate	ampli- tude	rate	ampli- tude	
Nov. 12 24	0.01	11	0	39	64	34	70	40	54			Single beats are blocked. Escapes after missing a few beats. Inhibition occurred at intervals. Inhibition only after return of Ringer's. No change in sinus rhythm on second perfusion but complete ventricle pause. Second perfusion not effective. Paralysis of center?
	1a.1	5	0	36	27	28	35	35	26			
	1b.1	12	0	31	26	31		32	26			
Dec. 4	0.1	0	0	20	32	11	50	24	24			Second test nil. Inhibition when Ringer's perfusion stopped. Second test nil. Single beats missed. Second test nil. Paralysis? Blocks at intervals. Released when vagi cut. Released by cutting right vagus. Electrical stimulation of left vagus caused inhibition; re- covery gradual. Anemia during recovery produced inhibition; re- covery gradual. Left vagus cut, no effect. Right vagus cut, re- leased the heart. Escapes every third beat. Block? Inhibition late after return to Ringer's. Sinus rhythm slowed only. Block increased by anemia in Ringer's. Sinus beating. Second test same result. Second 12 min. perfusion gave no inhibition. Central paralysis.
	0.1	3	10	31	40	Inh.	0	33	47			
5	0.1	3	10	31	40	Inh.	0	33	47			Second test nil. Inhibition when Ringer's perfusion stopped. Second test nil. Single beats missed. Second test nil. Paralysis? Blocks at intervals. Released when vagi cut. Released by cutting right vagus. Electrical stimulation of left vagus caused inhibition; re- covery gradual. Anemia during recovery produced inhibition; re- covery gradual. Left vagus cut, no effect. Right vagus cut, re- leased the heart. Escapes every third beat. Block? Inhibition late after return to Ringer's. Sinus rhythm slowed only. Block increased by anemia in Ringer's. Sinus beating. Second test same result. Second 12 min. perfusion gave no inhibition. Central paralysis.
6	0.1	3	15	30	35	Inh.	0	31	35			
8	0.1	3	40	19	40	17	43	21	26			Second test nil. Inhibition when Ringer's perfusion stopped. Second test nil. Single beats missed. Second test nil. Paralysis? Blocks at intervals. Released when vagi cut. Released by cutting right vagus. Electrical stimulation of left vagus caused inhibition; re- covery gradual. Anemia during recovery produced inhibition; re- covery gradual. Left vagus cut, no effect. Right vagus cut, re- leased the heart. Escapes every third beat. Block? Inhibition late after return to Ringer's. Sinus rhythm slowed only. Block increased by anemia in Ringer's. Sinus beating. Second test same result. Second 12 min. perfusion gave no inhibition. Central paralysis.
9	0.1	1	15	21	50	4	Inc.	27	28			
10	0.1	4	0	33	45	Inh.	0	30	38			Second test nil. Inhibition when Ringer's perfusion stopped. Second test nil. Single beats missed. Second test nil. Paralysis? Blocks at intervals. Released when vagi cut. Released by cutting right vagus. Electrical stimulation of left vagus caused inhibition; re- covery gradual. Anemia during recovery produced inhibition; re- covery gradual. Left vagus cut, no effect. Right vagus cut, re- leased the heart. Escapes every third beat. Block? Inhibition late after return to Ringer's. Sinus rhythm slowed only. Block increased by anemia in Ringer's. Sinus beating. Second test same result. Second 12 min. perfusion gave no inhibition. Central paralysis.
13a	0.01	8	0	31	26	Inh.	0	14	31			
13b	0.01	8	25	36	18	Inh.	0	37	14			Second test nil. Inhibition when Ringer's perfusion stopped. Second test nil. Single beats missed. Second test nil. Paralysis? Blocks at intervals. Released when vagi cut. Released by cutting right vagus. Electrical stimulation of left vagus caused inhibition; re- covery gradual. Anemia during recovery produced inhibition; re- covery gradual. Left vagus cut, no effect. Right vagus cut, re- leased the heart. Escapes every third beat. Block? Inhibition late after return to Ringer's. Sinus rhythm slowed only. Block increased by anemia in Ringer's. Sinus beating. Second test same result. Second 12 min. perfusion gave no inhibition. Central paralysis.
17	0.1	14	0	23	32	9	43	24	20			
20	0.1	8	40	27	41	4	48	31	35			Second test nil. Inhibition when Ringer's perfusion stopped. Second test nil. Single beats missed. Second test nil. Paralysis? Blocks at intervals. Released when vagi cut. Released by cutting right vagus. Electrical stimulation of left vagus caused inhibition; re- covery gradual. Anemia during recovery produced inhibition; re- covery gradual. Left vagus cut, no effect. Right vagus cut, re- leased the heart. Escapes every third beat. Block? Inhibition late after return to Ringer's. Sinus rhythm slowed only. Block increased by anemia in Ringer's. Sinus beating. Second test same result. Second 12 min. perfusion gave no inhibition. Central paralysis.
22	0.1	4	0	23	31	16	36	25	33			
Feb. 13a	0.1	0	54	22	40	8	65	22	42			Second test nil. Inhibition when Ringer's perfusion stopped. Second test nil. Single beats missed. Second test nil. Paralysis? Blocks at intervals. Released when vagi cut. Released by cutting right vagus. Electrical stimulation of left vagus caused inhibition; re- covery gradual. Anemia during recovery produced inhibition; re- covery gradual. Left vagus cut, no effect. Right vagus cut, re- leased the heart. Escapes every third beat. Block? Inhibition late after return to Ringer's. Sinus rhythm slowed only. Block increased by anemia in Ringer's. Sinus beating. Second test same result. Second 12 min. perfusion gave no inhibition. Central paralysis.
13b	0.1	2	0	22	42	22	42	26	36			

14	0.1	2	55	19	38	Inh.	0	21	41	Immediate release on cutting right vagus. Figure 2.
14	0.1	3	45	36	42	Inh.	0	36	41	Broke through. No second inhibition. Central paralysis?
19	0.1	7	50	44	44	7	64	44	43	Right vagus cut, release.
19	0.1	4	30	27	45	Inh.	0	28	36	Broke through.
20	0.1	3	15	24	36	Inh.	0	24	36	Broke through.
20a	0.01	6	0	27	55	Inh.	0	27	52	Left vagus cut, no effect. Slow recovery. Figure 4.
20b	0.01	6	50	26	53	9	55	28	52	Irregular. Recovery only after cutting right vagus. Figure 3.
21a	0.01	7	40	26	43	Inh.	0	25	45	Second inhibition total. Left vagus cut, no release. Figure 1.
21b	0.01	10	40	28	48	Inh.	0	36	44	Sinus beating regularly during ventricular inhibition. Ventricle escaped after 9 minutes.
Apr. 4a	0.1	3	30	34	38	6	Inc.	35	37	Sinus beating regularly. Block?
4b	0.05	11	40	43	47	16	Inc.	43	45	Block to 3-1 rhythm.

THE INFLUENCE OF SOME DRUGS USED IN THE TREATMENT OF GOUT (AND ARTHRITIS) ON THE ELIMINATION OF URIC ACID AND OTHER WASTE PRODUCTS FROM THE BLOOD

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For many years clinicians have striven to find some drug the administration of which would enable the gouty organism to get rid of its excess of uric acid. As examples of such therapeutic practices may be mentioned the use of the salts of benzoic, salicylic, and of quinic acid, of colchicum, of lithium citrate and of late years of phenyl quinolin carbonic acid (atophan).

On account of our ignorance concerning the etiology of gout it is difficult to decide at this time whether drugs used in the treatment of this disease owe their favorable effect to a power possessed by them of causing a rapid excretion of uric acid; or to some other pharmacological action. The power of producing an increased uric acid elimination in the urine has been ascribed to a large number of substances, and in the case of two important drugs of this class, salicylic and phenyl quinolin carbonic acid (atophan) both of which have been extensively used in the treatment of gout, it has been recently shown¹ that this increased excretion is accompanied by an increased elimination of uric acid from the blood. Whether this diminution of circulating uric acid has in itself any favorable effect on gout it is difficult to say, as it is perfectly conceivable that many substances absolutely devoid of therapeutic power might produce this result. In order to test this latter point I have carried

¹ Folin and Lyman: *This Journal*, 1913, iv, 539. Fine and Chace: *Soc. Exp. Biol. and Med.*, 1915, xii, 95. *Ibid.*: *Journ. Biol. Chem.*, 1915, xxi, 371. Denis: *This Journal*, 1915.

out a series of experiments with a number of substances supposed to influence uric acid excretion, benzoic, cinnamic, quinic, and paraoxybenzoic acids, and colchium.

In an attempt to answer the question as to whether the effect of these drugs on the kidney is selective for uric acid or whether they also cause an increased elimination from the blood of other substances I have also made a few experiments dealing with the effect of benzoic acid, salicylic acid, aspirin (acetyl salicylic acid), and atophan on the creatin content of blood.

In this work it would of course have been desirable to have used only gouty individuals showing uric acid retention in the blood; such subjects are however very difficult to secure in sufficient numbers, and as we now know that uric acid retention, as shown by blood analysis, sometimes occurs in individuals having no clinical symptoms of gout, it seemed best to carry out the experiments on such easily obtainable subjects as convalescent patients and on those suffering from minor illnesses.

All the subjects used in the following experiments were adult patients in the medical wards of the Massachusetts General Hospital. In every case they were placed on a purin free diet at least one or two days before any observations were made and were kept on this diet during the entire experimental period.

The quantity of the drug to be administered each day was divided into three equal portions and was given dissolved in about three hundred cubic centimeters of water immediately after meals.²

EFFECT OF SODIUM BENZOATE ON THE URIC ACID ELIMINATION

The older observers claimed that the ingestion of benzoic acid or its salts caused an increased excretion of uric acid in the urine. On account of its importance as a food preservative benzoic acid has within recent years been the subject of much experimental work,³ as a result of which we now know that

² The methods of analysis used were the same as those employed in the first paper on this subject (this Journal, 1915, vii, p. 255). Creatin in blood was determined by the method of Folin (Jour. Biol. Chem., 1914, xvii, 463.)

³ Wiley: Bull. 84, part 4, Bureau of Chemistry, U. S. Dept. of Agriculture. Chittenden, Long, Herter, Report 88, U. S. Dept. of Agriculture.

with normal men the ingestion of as much as four grams of benzoic acid per day causes no increase in the output of uric acid; indeed Long states that one subject was able to take as much as 10 grams of sodium benzoate per day without increasing the elimination of the former substance.

As will be seen from the following results benzoic acid, like salicylic acid, causes when taken in large doses, a marked decrease in the uric acid content of the blood, and an increase in the uric acid content of the urine. This effect, however, is not so constant or so marked as with the former drug. In one case in which a daily dose of only 5 grams was administered for two days to a woman weighing 90.9 kilos no change in the uric acid content of the blood was found; also in one case (Experiment III) when 8 grams was administered for three days to a large man no change was noted in the uric acid content of blood or urine.

Experiment I. D. 7. Male, 40 years old, weight 56.7 k. Hodgkin's disease, early stage.

BLOOD (100 GRAMS)				URINE	
Day	Sodium benzoate	Non-protein nitrogen	Uric acid	Nitrogen	Uric acid
	grams	mgm.	mgm.	grams	gram
1.....	0	30	1.6	9.3	0.48
2.....	0			9.4	0.51
3.....	8			11.2	0.58
4.....	8			11.0	0.72
5.....	8	28	0.7	9.9	0.69
6.....	0			7.1	0.48

Experiment II. D. L. 31. Male, 38 years old, weight 54 k. Convalescent from lung abscess.

BLOOD (100 GRAMS)				URINE	
Day	Sodium benzoate	Non-protein nitrogen	Uric acid	Nitrogen	Uric acid
	grams	mgm.	mgm.	grams	gram
1.....	0	36	1.2	5.2	0.48
2.....	8			8.1	0.51
3.....	8			6.2	0.60
4.....	8	30	0.6	8.4	0.47

Experiment III. J. W. 31. Male, 58 years old, weight 85.4 k. Myocardial weakness, arteriosclerosis.

BLOOD (100 GRAMS)				URINE	
Day	Sodium benzoate	Non-protein nitrogen	Uric acid	Nitrogen	Uric acid
	grams	mgm.	mgm.	grams	gram
1.....	0	27	1.9	10.8	0.80
2.....	8			7.0	0.78
3.....	8			11.0	0.82
4.....	8	28	2.0	11.4	0.78

TABLE I
Blood (100 grams)

DAY	SODIUM BENZOATE	NON-PROTEIN NITROGEN	URIC ACID	
	grams	mgm.	mgm.	
1.....	0	39.0	1.6	B. E. 31. Male, 22 years old. Weight 45 k. Gonorrheal arthritis
2.....	8			
3.....	8			
4.....	8			
5.....	0	40.0	0.6	
1.....	0	40.0	2.0	G. O. 31. Male, 36 years old. Weight 79.5 k. Subacute arthritis. Mitral stenosis
2.....	8			
3.....	8			
4.....	8			
5.....	0	44.0	1.3	
1.....	0	32.0	2.1	G. R. 31. Male, 38 years old. Weight 63.3 k. Chronic infectious arthritis
2.....	8			
3.....	8			
4.....	8			
5.....	0	40.0	0.6	
1.....	0	24.0	3.7	W. A. 16. Female, 47 years old. Weight 90.9 k. Subacute arthritis (gonorrheal)
2.....	5			
3.....	5	25.0	3.5	

THE EFFECT OF SODIUM CINNAMATE ON THE URIC ACID ELIMINATION

In former years cinnamic acid was employed to a limited extent in the treatment of rheumatic fever and of chronic arthritis. Its "antirheumatic" action was however found to be small compared to that possessed by salicylic acid and its derivatives so that it is now little used. Cinnamic acid is said to cause an increased excretion of uric acid in the urine, and in view of its undoubted transformation into benzoic acid in the body it is safe to assume that its action is probably similar to that obtained with the latter substance.

As will be seen from the results presented in Experiment V cinnamic acid when taken in doses of 6 grams per day for three days produced a slightly increased uric acid output in the urine and a decrease in the uric acid content of the blood. Smaller doses (Experiment IV) have no such effect.

It would have been of interest to have observed the effect of the administration of larger doses of sodium cinnamate but its very nauseating taste made it necessary to abandon this line of work.

Experiment IV. D. E. Normal female, 36 years old. Weight 90.9 k.

BLOOD (100 GRAMS)				URINE	
Day	Sodium cinnamate	Non-protein nitrogen	Uric acid	Nitrogen	Uric acid
	grams	mgm.	mgm.	grams	gram
1.....	0	29	1.6	7.0	0.56
2.....	4			6.5	0.54
3.....	4	28	1.8	7.5	0.51
4.....	0			7.4	0.51

Experiment V. U. L. 31. Male, 19 years old, weight 65.0 k. Convalescent from lobar pneumonia.

Day	BLOOD (100 GRAMS)			URINE	
	Sodium cinnamate	Non-protein nitrogen	Uric acid	Nitrogen	Uric acid
	grams	mgm.	mgm.	grams	gram
1.....	0	28	2.0	9.7	0.48
2.....	6			9.5	0.76
3.....	6				
4.....	6	29	1.4	8.9	0.50

THE EFFECT OF SODIUM QUINATE ON THE URIC ACID EXCRETION

Quinic acid (hexahydroxytetra oxy benzoic acid) is transformed in the body into benzoic acid⁴ as shown by the large amounts of hippuric acid excreted when it is ingested. Whether this transformation is quantitative is however not known. As to the effect of quinic acid on the uric acid excretion varying results have been reported.⁵ Weiss claimed that the ingestion of quinic acid caused a marked decrease in the uric acid excretion, a decrease due in his opinion to a decreased formation of this body, and on the basis of this work lithium quinate, piperazine quinate, etc., were highly recommended some ten or more years ago as being efficacious in the treatment of gout. Later workers,⁶ however, have been unable to find any change in the output of uric acid even when quinic acid was administered in doses of 25 grams per day.

In the two experiments made with this acid no change in the uric acid content of the blood or in the excretion of uric acid was noted.

⁴ Stadelmann: Arch. f. Exp. Path. u. Pharm., 1879, x, 317. Hupfer: Zeit. f. Physiol. Chem., 1902, xxxvii, 302.

⁵ Zeit. f. Physiol. Chem., 1898, xxv, 393. Berliner Klin. Wochenschr., 1899, No. 14.

⁶ Hupfer: Zeit. Phys. Chem., 1904, xxvii, 203. Lewandouski: Zeit. f. Klin. Med., 1900, Heft 3 and 4. Ulrici: Arch. f. Exp. Path. und Pharm., 1900, xlii, 321.

Experiment VI. F. i. 31. Male, 28 years old, weight 65.9 k. Convalescent after a mild attack of lobar pneumonia.

BLOOD (100 GRAMS)				URINE	
Day	Sodium quinate	Non-protein nitrogen	Uric acid	Nitrogen	Uric acid
	<i>grams</i>	<i>mjm.</i>	<i>mjm.</i>	<i>grams</i>	<i>gram</i>
0.....	0	24	1.6	16.0	0.51
1.....	6.5			17.5	0.54
2.....	9				
3.....	9	30	1.6	14.7	0.54
				12.7	0.50

Experiment VII. Th. 31. Male, 40 years old, weight 60.9 k. Peptic ulcer. Convalescent.

BLOOD (100 GRAMS)				URINE	
Day	Sodium quinate	Non-protein nitrogen	Uric acid	Nitrogen	Uric acid
	<i>grams</i>	<i>mjm.</i>	<i>mjm.</i>	<i>grams</i>	<i>gram</i>
1.....	0	38	1.3	7.9	0.59
2.....	7.6			9.1	0.59
3.....	11			10.2	0.56
4.....	0	35	1.2		

THE EFFECT OF PARAOXYBENZOIC ACIDS ON THE URIC ACID ELIMINATION

The homologues of salicylic acid, meta and paraoxybenzoic acid have been shown to have absolutely no effect when administered to persons suffering from rheumatic fever, arthritis or gout.⁷ It is therefore of interest to determine whether these substances have any effect on the blood.

From the results of the two experiments given below it would seem that paraoxybenzoic acid, a substance apparently of no therapeutic value does not possess the power so strikingly shown by its homologue, salicylic acid, of increasing uric acid elimination from the blood. As in the earlier experiment of Rockwood⁸ no increase of uric acid elimination in the urine was observed.

⁷ Stockman: British Med. Journ., 1913, i, 597.

Experiment VIII. McL. 31. Male, 45 years old, weight 58.1 k. Cholelithiasis.

BLOOD (100 GRAMS)				URINE	
Day	Paraoxy-benzoic acid	Non-protein nitrogen	Uric acid	Nitrogen	Uric acid
	grams	mgm.	mgm.	grams	gram
1.....	0	34	1.8	7.5	0.32
2.....	5			7.0	0.35
3.....	6	.		11.6	0.37
4.....	8	30	1.8	9.5	0.36

Experiment IX. Sp. 31. Male, 25 years old, weight 59.6 k. Infarction of spleen.

BLOOD (100 GRAMS)				URINE	
Day	Paraoxy-benzoic acid	Non-protein nitrogen	Uric acid	Nitrogen	Uric acid
	grams	mgm.	mgm.	gram	gram
1.....	0		1.6	9.2	0.63
2.....	3	40.0		7.3	0.61
3.....	8			10.0	0.58
4.....	8	40.0	1.7	11.1	0.55

THE EFFECT OF COLCHICUM ON THE URIC ACID EXCRETION

Concerning the effect of colchicum on the uric acid elimination various conflicting reports have been made, some observers claiming that it produces an increased uric acid excretion, others that it causes no change.⁸

As colchicum is one of the oldest remedies for gout and is still recommended by many clinicians as being of great efficiency in the treatment of this disease it has seemed worth while to include in this series of experiments a few observations on the effect of this drug on the uric acid elimination.

⁸ American Journ. Physiol., 1909, xxv, 34.

⁹ Jacoby: Arch. f. Exp. Path. u. Pharm., xxvii, p. 119. Fancett: Guy's Hospital Reports, lii, p. 115. Paton: British Med. Journ., 1886, i, p. 377. Ibid.: Journ. Anat. u. Physiol., xx, p. 267.

I have administered colchicum to five individuals and have in no case obtained any appreciable increase in the uric acid excretions in the urine. In three cases the drug produced no change in the uric acid content of the blood, in two cases a slight increase was obtained.

Experiment XI. Sp. Male, 22 years old, weight 65 k. Purpura.

BLOOD (100 cc.)				URINE	
Day	Wine of colchicum	Non-protein nitrogen	Uric acid	Nitrogen	Uric acid
	cc.	mgm.	mgm.	grams	gram
1.....	0	35	2.2	7.3	0.48
2.....	0			7.6	0.50
3.....	6			6.8	0.48
4.....	6			7.4	0.46
5.....	6	35	2.0	8.0	0.44

Experiment XII. C. O. 7. Male, 22 years old, weight 57 k. Convalescent after tonsillectomy.

BLOOD (100 GRAMS)				URINE	
Day	Wine of colchicum	Non-protein nitrogen	Uric acid	Nitrogen	Uric acid
	cc.	mgm.	mgm.	grams	gram
1.....	0	30.0	1.6	7.3	0.29
2.....	4.0			7.6	0.30
3.....	6.0			6.8	0.28
4.....	6.0			7.4	0.26
	6.0	38.0	2.0	12.1	0.27

Experiment XIII. Ch. 31. Male, 28 years old, weight 60 k. Abdominal tumor.

BLOOD (100 cc.)				URINE	
Day	Wine of colchicum	Non-protein nitrogen	Uric acid	Nitrogen	Uric acid
	cc.	mgm.	mgm.	grams	gram
1.....	0	30	1.2	12.2	0.39
2.....	6			11.0	0.39
3.....	6			10.1	0.42
4.....	6	31	1.3	12.0	0.36
5.....	0			10.9	0.39

Experiment XIV. C. B. 31. Male, 26 years old, weight 63 k. Osteomyelitis of femur.

BLOOD (100 cc.)				URINE	
Day	Wine of colchicum	Non-protein nitrogen	Uric acid	Nitrogen	Uric acid
	cc.	mgm.	mgm.	grams	gram
1.....	0	28	1.6	10.2	0.48
2.....	6			11.3	0.52
3.....	6			12.0	0.44
4.....	6	26	1.7	9.0	0.44
5.....	0			11.8	0.50

Experiment XV. J. H. 31. Male, 20 years old, weight 55 k. Hemiplegia.

BLOOD (100 cc.)				URINE	
Day	Wine of colchicum	Non-protein nitrogen	Uric acid	Nitrogen	Uric acid
	cc.	mgm.	mgm.	grams	gram
1.....	0	35	1.1	7.6	0.40
2.....	6			9.0	0.40
3.....	6			10.1	0.42
4.....	6	35	1.3	9.9	0.42
5.....	0			8.7	0.41
6.....	0			8.4	0.40

THE EFFECT OF SODIUM BENZOATE, ASPERIN AND ATOPHAN ON THE CREATIN ELIMINATION

Sodium benzoate, asperin and atophan when given in sufficiently large amounts cause an increased elimination of uric acid from the blood, but as will be seen by the following results those substances have practically no effect on the creatin content of the blood, a not unexpected finding in view of the results recently published by Folin and Denis¹⁰ in which it was shown that a retention of creatin occurs only in cases of extremely marked kidney inefficiency.

The results presented above lend some support to the view that the good effects produced by the drugs at present considered

¹⁰ Journ. Biol. Chem., 1914, xxvii, 482.

TABLE II
Blood (100 grams)

DAY	SODIUM BENZOATE	NON- PROTEIN NITROGEN	CREATINE	
	<i>grams</i>	<i>mgm.</i>	<i>mgm.</i>	
1.....	0	38	9.0	Fr. 16. Female, 40 years old. Weight 65 k. Abdominal tumor
2.....	5.3			
3.....	5.3	36	9.0	
1.....	0	35	9.0	McG. 16. Female, 60 years old. Weight 60.9 k. Abdominal tumor
2.....	5.3			
3.....	8.0			
4.....	5.3	38	9.0	
1.....	0	40	9.0	J. 16. Female, 31 years old,. Weight 58.1 k. Chronic arthritis
2.....	4			
3.....	4			
4.....	4			
5.....	4			
6.....	4			
7.....	4	30	8.0	
1.....	0	40	8.5	M. 16. Female, 58 years old. Weight 50.0 k. Subacute arthritis
2.....	8			
3.....	8			
4.....	8			
5.....	8			
6.....	8	45	7.2	

DAY	ASPERIN	NON- PROTEIN NITROGEN	CREATIN AND CRE- ATININE	URIC ACID	
	<i>grams</i>	<i>mgm.</i>	<i>mgm.</i>	<i>mgm.</i>	
1.....	0	30	10.0	2.0	D. M. 7. Male, 31 years old. Weight 50.4 k. Chronic arthritis
2.....	7				
3.....	10				
4.....	4.7				
5.....	4.7				
6.....	4.7	32	11.0	0.7	
	ATOPHAN				
1.....	0	40	8.5		Ro. 7. Male, 37 years old. Weight 68.1 k. Sciatica
2.....	1				
3.....	3				
4.....	3				
5.....	3				
6.....	3				
7.....	3	33	10.0		

by clinicians as most efficient in the treatment of gout may in part at least be due to a power possessed by them of producing a lowered kidney threshold for certain specific substances. Atophan and the salicylates possess this power in a marked degree and have, according to many clinicians, marked therapeutic effects. Sodium benzoate long known to have but little "antirheumatic" effect possesses the power of increasing elimination to a somewhat smaller extent, while cinnamic, quinic and paraoxybenzoic acids now known to be devoid of therapeutic action also apparently do not influence the elimination. The results obtained with colchicum do not, however, lend themselves to the above interpretation.

SUMMARY

It has been shown that benzoic acid when administered to men in large doses (8 grams per day) increases the uric acid excretion in the urine and decreases the uric acid content of the blood. Cinnamic acid in the comparatively small doses used (4-6 grams per day) had little if any effect. Quinic acid and colchicum have also no effect on the uric acid elimination. The same is true of paraoxybenzoic acid which shows none of the power possessed by its homologue, salicylic acid, of producing a lowered kidney threshold for uric acid. None of these drugs produce any change in the non-protein nitrogen content of the blood. Benzoic acid, aspirin, and atophan were found to have no effect on the creatin content of the blood.

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